Volume XXVII January, 1929 Number 1

SOIL SCIENCE

Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

SOIL SCIENCE

VOLUME XXVII

JANUARY - JUNE, 1929

RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY
U. S. A.

PUBLISHED BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MARYLAND

CONTENTS

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to Dr. Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

Soil Science is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Volumes begin with the January and July issues, respectively. The subscription price is $10.00 for Volumes XXV (Nos. 1–6 inclusive) and XXVI (Nos. 1–6 inclusive) January, 1928, to December, 1928, inclusive, United States, Mexico, Cuba. An additional charge per volume of 50 cents for subscriptions in other countries. *Back volumes* are supplied on orders for Vols. I to XIV inclusive, XIX to XXIV incl.—$100.00 per set.

New subscriptions are accepted to begin with the current issue, unless the subscriber gives instructions to begin the subscription with some other issue. If the subscriber desire, back issues of the current volume may be obtained when in stock. The publishers do not guarantee to supply back issues, however.

Renewal subscriptions. To avoid a break in your series, subscriptions should be renewed promptly on expiration. Otherwise it may prove impossible for the publishers to furnish the back issues missed, as only sufficient copies are printed of each issue to supply orders in hand.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cía, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; or G. Jervis Manton, 430 Lit. Collins St., Melbourne (and at Sydney and Brisbane)

For the British Empire, except Australia and Canada: Bailliere, Tindall & Cox; 8 Henrietta Street, Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann, Krausenstrasse 38, Berlin N. W., Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

Publishers of: *Medicine, Journal of Urology, Bulletin of the Johns Hopkins Hospital, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Physical Therapeutics, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thompson Research Laboratory, Journal of Biological Chemistry, Chemical Reviews. Journal of Dairy Science, Soil Science, Social Forces, Personnel Journal, Journal of Comparative Psychology. Comparative Psychology Monographs, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, American Speech, Childhood Education.*

A STUDY OF THE NATURE OF THE NITROGENOUS COMPOUNDS IN FUNGOUS TISSUE AND THEIR DECOMPOSITION IN THE SOIL

A. FLOYD HECK

University of Wisconsin[1]

Received for publication August 16, 1928

The decomposition of organic matter in nature, especially the cellulosic portion, is largely accomplished by fungous activities and large amounts of mycelial tissue are formed. The fungous material is relatively high in nitrogen, which is obtained from the substrate, for the most part in the mineral form. As a result, much soluble inorganic nitrogen is changed to an insoluble organic form in the mycelial tissue. The nature of this nitrogen and the factors which govern its return to the inorganic form are made the subject of this study.

OCCURRENCE AND QUANTITY OF SOIL FUNGI

Mold growth in the soil has been observed for a long time. Perhaps Adametz (4) in 1886 was one of the first to isolate molds from the soil. Sixteen years later, in 1902, Oudemaus and Köning (78) isolated and described some 45 species, and thus the first real study of soil fungi began. Following these early workers, the greater part of the investigations with soil fungi has been in relation to systematic studies. Jensen (58), Dale (23, 24), Waksman (109), and Abbott (1, 2, 3) have classified as many as 200 species of fungi living in the soil. Of this large number of species, those of *Penicillium*, *Aspergillus*, *Mucor*, *Trichoderma*, *Cladisporium*, *Fusarium*, and *Rhizopus* are found most often. More recently Gilman and Abbott (41) have compiled a summary of the soil fungi in which is gathered together most of the available material on their classification.

Because of more favorable conditions with respect to oxygen and organic matter, soil fungi are found chiefly in the surface soil. Waksman (108) found few fungi below a depth of 12 to 20 inches, and on the plots at Rothamsted, Brierley (89) reported a rapid diminution in number as the depth increased. On the other hand Rathbun (86) reported fungi to a depth of 44 inches but believed that grubs and worms were in part responsible for this distribution.

[1] From the Departments of Agricultural Bacteriology and Soils. This work was supported in part by a grant from the special research fund of the University of Wisconsin. For the many valuable suggestions and criticisms received in the course of this investigation, the writer wishes to express his sincere gratitude to Dr. E. B. Fred and to Prof. A. R. Whitson, under whose supervision this work was done.

Neither climatic nor soil conditions seem to be deciding factors in the distribution of fungi, for Pratt (81) isolated many of the lower forms from the desert soils of southern Idaho, and Paine (79) found the same groups to predominate in virgin soils. Geographically, the distribution of soil fungi is almost universal, for Waksman (109) isolated the same species in soils from North America, Europe, and the Hawaiian Islands.

As it is impossible to count the soil fungi *in situ*, their estimation is difficult as well as inaccurate. The dilution method has often been used, but this at best gives only a rough estimate of the number of spores and very little idea as to the actual amount of fungous tissue in the soil. The plate count for soil fungi has been given as ranging from a few thousands to one or two millions per gram of soil, varying with the conditions. Brierley (89) found a sort of seasonal rhythm on the manured plot at Broadbalk, the count being high in March or April and September or October and low in July and December. Waksman (112) showed that the presence of manure or other energy material increased the number of soil fungi. He found a range of 151,000 in a poor forest soil to 525,000 in a garden soil and 750,000 in a meadow soil. Waksman and Starkey (121) have shown that organic matter or energy material added to the soil increases the number of microorganisms. They reported that dextrose increases the number of bacteria, and cellulose the number of fungi in the soil. Rye straw acts very much like cellulose.

Conn (21, 22) and Waksman (112) have shown that fungi exist in the soil in the mycelial form as well as in the spore stage, and McLennan (71) has found that the plate counts are not correlated with the vegetative growth. If the conditions for vegetative growth are good, fewer spores are formed than under more adverse conditions and so the plate count shows a lower number under the better conditions, although in reality a greater quantity of fungous tissue is present. The amount of fungous material present in a soil, therefore, has little relation to the plate count. Brierley (89) estimated that there is about 60 pounds per acre of dry fungous material in the surface soil and that this carries approximately 6 pounds of nitrogen.

ACTIVITIES OF SOIL FUNGI

Relation to energy materials

Fungi are non-chlorophyll bearing plants and must obtain their energy from materials already synthesyzed. This they do by means of enzymes which they secrete. A great many workers (18, 27, 28, 31, 83, 90, 92, 94, 111, 135) have investigated the enzymes of various fungi and have found that practically all of the common organic compounds are hydrolyzed by enzymes secreted by the fungi. The common sugars are readily utilized. It has been shown by several investigators (25, 45, 46, 64, 80, 87, 93) that the pentose sugars and the pentosans in organic materials are used as sources of energy by various groups of the fungi. Cellulose is one of the more resistant substances found

in nature and was originally believed to be hydrolyzed microbiologically only by the bacteria. A number of later workers (46, 48, 50, 69, 75, 91, 103, 118, 120) have shown, however, that cellulose, either in the pure form or in the natural or crude state found in plant residues such as straw, roots, or bagasse, is hydrolyzed by filamentous fungi and used by them as a source of energy. It has also been shown (118) that nitrogen is necessary in this process for the metabolism of the organisms. Lignin is probably one of the most resistant substances found in organic residues. Waksman and Tenney (119) found the lignin unattacked by most soil microorganisms and were able to recover it almost quantitatively at the end of a 35-day incubation period. On the other hand, von Schrenk (94) has shown that even lignin is hydrolyzed and destroyed by the white rot fungus *Polyporus juniperinus*. Other substances such as β-methylglucoside (36) and higher alcohols (76) have been shown to be used as sources of energy by fungi.

Natural organic materials in the form of plant residues probably contain many if not all of these carbonaceous substances which are used as energy for the growth of the fungi. Wheat straw, for example, according to Waksman (113), contains 21.67 per cent pentosans, 34.27 per cent cellulose, and 21.21 per cent lignin. It also contains water-soluble carbonaceous materials, and so furnishes an excellent source of energy for the fungi.

Relation of fungi to nitrogen

Soil microorganisms are concerned with nitrogen in three ways: first, the assimilation of free nitrogen; second, the transformation of organic to inorganic forms; third, the use of combined nitrogen in the production of cellular or mycelial substances.

Fixation of free nitrogen. Among the early workers on the fixation of free nitrogen by soil fungi was Lipman (66) who reported a weak fixing power for species of *Aspergillus* and *Penicillium*. Later workers (17, 42, 63), however, seem to be of the opinion that with the possible exception of *mycorrhiza*, there is no fixation of atmospheric nitrogen by soil fungi. Although Duggar and Davis (37) concurred in the general opinion of the latter workers their work seems to indicate that *Phoma betae* has a weak nitrogen fixing power.

Ammonification. The power of soil fungi to hydrolyze nitrogenous organic materials, especially the proteins and amino compounds found in the organic matter, has been investigated by a number of workers (1, 62, 70, 108, 110, 116) who have shown this property to be very general among the fungi. A great many of the fungi have the power to break down the proteins and to liberate amino acids, or ammonia, or both. Thus the soil fungi play a very important rôle in this stage of the mineralization process.

Use of nitrogen. It is generally conceded by most investigators (9, 12, 19, 60, 82, 118) that nitrogen is not only used by the fungi in their growth but is also a necessary element in the formation of their substance. This nitrogen they may use in the inorganic form as nitrate, nitrite, or ammonia, or in the

various amino forms. Reduction of nitrogen has been shown, but there is very little evidence of denitrification or the loss of combined nitrogen from fungous activities. The use of nitrogen by fungi is always associated with the use of energy material and it has been pointed out (8, 16, 19, 48, 87, 98, 120) that this nitrogen, if not an absolute necessity in the decomposition of the cellulosic energy material, is an aid in this process and hastens the utilization of the cellulose by the fungi. The amount of nitrogen necessary in this decomposition process has been estimated by Waksman and Heukelekian (47, 117, 118) to be 1 part of nitrogen for every 30 to 50 parts of energy material in the form of cellulose used by the fungi. This nitrogen is used in the building of mycelial tissue, and the amount consumed will vary somewhat with the organisms and its environmental conditions.

The result of this use of nitrogen in relation to the utilization of energy material by microorganisms is seen under field conditions in the depression of nitrate nitrogen when the energy material of the soil is increased. A number of workers (5, 68, 74, 85, 95, 97, 99) have reported that when energy material in the form of plant residue, either tops or roots, is incorporated into the soil there is an immediate depression in the nitrate content. At first toxins were thought to be the cause of this depression but later investigations (13, 20, 67, 106) seem to indicate that it is only a disturbance of the energy-nitrogen balance and that the nitrogen is not lost, but is used by the soil microorganisms.

As a brief summary it may be stated that the soil fungi use the carbonaceous materials of the soil as energy for their growth and the soil nitrogen in building their mycelial tissue. If the ratio of energy material to nitrogen is decreased, soluble nitrogen is liberated, but if this ratio is increased, these organisms use up available nitrogen. It is then easy to believe that soil fungi are largely responsible for the rapid depression of mineral nitrogen when the energy supply of the soil is increased.

COMPOSITION OF FUNGOUS MYCELIUM[2]

The composition of fungous tissue has been the subject of investigation for over a century. Perhaps the outstanding single contribution is the monograph by Zellner (136) in which he has compiled much of this material, especially from the earlier works. The greater part of this information, however, is of a general qualitative nature and only a limited amount of quantitative data is given. The reason for this may be seen in the fact that not only the quantitative but even the qualitative composition of fungous tissue depends much upon the environmental conditions of the organism during its period of growth. Zellner has shown that as a group the fungi contain about the

[2] While this article was in press, a paper appeared by R. C. Thomas [Composition of fungus hyphae I. The *Fusaria*. *Amer. Jour. Bot.* 15: 537–547. (1928)] dealing with the composition of the hyphae of species of *Fusaria*. This author thinks that the outer covering of the hyphae is made up of a protein-pectic compound and a cellulose-fatty-acid complex with a basic skeleton of chitin.

same groups of compounds, with the possible exception of cellulose and lignin, as the higher plants. In the nitrogenous group they contain proteins of various kinds, amino acids, amines, basic nitrogenous and purine substances, urea, and lecithin, as well as certain toxic substances of an alkaloid nature. In the carbonaceous group may be mentioned glucose, trehalose, glycogen, pentosans, mycodextran, paraisodextran, inulin, viscosin, chitin, organic acids, fats, higher alcohols, and a cellulosic carbonaceous material of unknown composition.

Nitrogenous portion

Because of variability in culture media, methods of analysis and species employed, the analytical data available for fungous tissue are more or less unsatisfactory from a quantitative standpoint. The carbon content of fungous tissue is rather constant but its content of nitrogen is quite variable. Analyses reported by Sieber (96), Mazé (73), Peterson, Fred, and Schmidt (80), Heukelekian and Waksman (48), and the writer are given in table 1.

These data indicate that the ratio of carbon to nitrogen of the mycelium, although rather stable and usually falling between 7 and 10 to 1, depends somewhat on the carbon-nitrogen ratio of the medium upon which the mycelium is grown and may be greater than 20 to 1 where this ratio in the medium is very high. Waksman and Heukelekian (118) reported 45 per cent of carbon in the dry mycelium and a nitrogen content of from 4 to 8 per cent depending on the nitrogen content of the medium. From the results given, it appears that the nitrogen content of the mycelium decreases with a decrease in the nitrogen supply until it reaches about 2 per cent, after which a further decrease in the supply of nitrogen results in a decreased production of mycelium. The results of other workers are in accord with the data already given.

The nature of the nitrogenous substances in fungous tissue is not definitely known. The following estimates made by Winterstein and Reuter (132) on the tissue of *Boletus edulis* give some idea as to the kinds and amounts of the various substances:

	per cent
Moisture	10
Ether extract	4
(Fat 3.25 per cent, colesterin 0.5 per cent and lecithin)	
Alcohol extract	12
(Trehalose 3 per cent, sugar, lecithin, trimethyl-histidine, adenine, guanine, hypoxanthine, choline, alanine, leucine, purine bodies, bases, etc. 9 per cent)	
Water extract	28
(Glycogen 5 per cent, trehalose, purine bodies, bases, amino acids, ash, etc., 23 per cent)	
Residue	46
(Protein 30 per cent, amorphous carbohydrates [paraisodextran] 10 per cent, and chitin 6 per cent)	

In *Agaricus campestris*, Winterstein and associates (133) estimated that 51.9 per cent of the total nitrogen is protein nitrogen, 7.8 per cent basic nitro-

TABLE 1

Relation of carbon and nitrogen in fungous tissue

INVESTIGATOR	ORGANISM	CULTURE AGE	MEDIA			MYCELIUM		
			Energy material	Nitrogen material	C/N ratio	Carbon	Nitrogen	C/N ratio
		days				*per cent*	*per cent*	
Sieber (1881)	*Penicillium* sp. and *Aspergillus glaucus*	75	Sugar	Gelatin	4.75	45.95	5.32	8.64
	Penicillium sp. and *Aspergillus glaucus*	75	Sugar	NH_4Cl	9.60	46.03	5.34	8.62
Mazé (1902)	*Eurotiopsis gayaui*	5	Sucrose	....	21.05	51.67	4.48	11.30
	Eurotiopsis gayaui	9	Alcohol	....	9.01	50.45	5.55	9.10
	Eurotiopsis gayaui	8	Glycerol	....	19.55	48.89	4.67	10.47
	Eurotiopsis gayaui	8	Lactic acid	....	20.00	51.51	4.73	10.89
Peterson, Fred and Schmidt (1922)	*Aspergillus niger*	7	Xylose	NH_4NO_3	22.86	44.5	4.5	9.90
	Aspergillus niger	9	Xylose	NH_4NO_3	22.86	46.9	4.6	10.20
	Aspergillus niger	14	Xylose	NH_4NO_3	22.86	46.0	4.2	10.95
	Aspergillus niger	28	Xylose	NH_4NO_3	22.86	45.4	4.2	10.81
	Aspergillus sp.	14	Xylose	NH_4NO_3	22.86	47.3	4.7	10.07
	Penicillium glaucum	9	Xylose	NH_4NO_3	22.86	50.7	5.7	8.90
	Penicillium glaucum	14	Xylose	NH_4NO_3	22.86	46.3	5.0	9.26
	Penicillium glaucum	29	Xylose	NH_4NO_3	22.86	49.7	5.9	8.42
This work (1928)	*Aspergillus oryzae*	14	Sucrose	NH_4NO_3	6.00	40.48	6.42	6.30
	Aspergillus oryzae	14	Sucrose	NH_4NO_3	30.00	41.10	3.83	10.70
	Aspergillus oryzae	14	Sucrose	NH_4NO_3	150.00	39.55	1.89	20.90
						mgm.	*mgm.*	
Heukelekian and Waksman (1925)	*Trichoderma* sp. in solution	17	Cellulose	$(NH_4)_2SO_4$	10.95	54.90	12.3	4.46
	Trichoderma sp. in solution	24	Cellulose	$(NH_4)_2SO_4$	10.95	78.5	18.2	4.31
	Trichoderma sp. in solution	31	Cellulose	$(NH_4)_2SO_4$	10.95	138.2	22.2	6.22
	Trichoderma sp. in solution	38	Cellulose	$(NH_4)_2SO_4$	10.95	138.3	23.7	5.83
	Trichoderma sp. in sand	7	Cellulose	NH_4NO_3	11.40	128.9	18.9	6.80
	Trichoderma sp. in sand	14	Cellulose	NH_4NO_3	11.40	283.3	27.4	10.30

gen, and 40.3 per cent amino acid nitrogen. Supporting these figures Yoshimura and Kania (134) reported 60.26 per cent of the nitrogen in *Cortinellus shiitake* as protein nitrogen, 2.13 per cent as ammonia nitrogen, and 37.61 per cent as non-protein nitrogen.

Besides the usual protein and amino acid compounds, urea has also been reported. Working with the mycelium of *Lycoperdon pyriforme*, Iwanoff (55) found that where the medium on which the organism is grown is high in nitrogenous and low in carbonaceous material, the total nitrogen of the mycelium is high (7.9 per cent) and the urea content may be as high as 4.3 per cent. When the reverse conditions are true the nitrogen content is low (4.3 per cent) and in this case no urea is found in the tissue. With *Lycoperdon molle* he found (51, 52) that the urea content increases very rapidly until just before the ripening stage, when it reaches its maximum, after which there is a sharp decline with usually little or no urea in the mature and ripened tissue. He (56, 57) also showed that *Psalliota campestris* may contain as much as 8.5 per cent of urea and that this organism is able to synthesize urea either from ammonium carbonate or from arginine.

Carbonaceous material

The carbonaceous matter seems to differ somewhat from the carbonaceous substances in ordinary plant materials, in that common sugar, starch, and lignin are not generally reported. Cellulose has been reported but there seems to be some question as to the exact nature of this substance in fungi.

In the tissue of *Boletus edulis*, Winterstein and Reuter (132) have reported trehalose, glycogen, and an amorphous carbohydrate, which they called "paraisodextran." Dox and Neidig (34) treated the mycelium of *Penicillium expansum* with hot water and extracted a polysaccharide which precipitated out as a white powder on cooling. This substances gives d-glucose on hydrolysis and seems to have the same constitution as starch or cellulose but is not hydrolyzed by amylase. They called this substance "mycodextran" and expressed the opinion that it acts as a reserve carbohydrate when the sucrose in the medium is exhausted. Dox (30) showed that with *Aspergillus niger* the content of mycodextran reaches its maximum between 5 and 7 days, or when autolysis begins, at which time it may be as much as 4.5 per cent or more. Dox and Neidig (35) isolated another polysaccharide from the mycelium of *Aspergillus niger* which on hydrolysis gave galactose. This they called "mycogalactan."

The five carbon compounds as a group seem to be found in only very small quantities. Wichers and Tollens (125) studied 10 species of wood-destroying fungi and reported pentosans ranging in amounts from 2.61 to 6.73 per cent by Kröber's method. Ishida and Tollens (49) studied five species and reported pentosans from 2.58 to 5.11 per cent. Dox and Neidig (33) worked with six *Aspergillus* and *Penicillium* species and found that the pentosans in no case exceed 1.17 per cent, which is much lower than for the higher forms

reported by Wichers and Tollens. Schmidt, Peterson, and Fred (93) reported only about 1 per cent of pentosans in molds and found very little variation in the amount whether the molds were grown on xylose or sucrose, whereas Rege (87) found 7.82 per cent in the aerial portion of a *Coprinus* sp. grown on rice straw.

Cellulosic material

The cellulosic portion of fungous mycelium has been studied for more than a century and yet little is definitely known as to its chemical constitution. As early as 1811 Braconnot (15) subjected the mycelium of certain of the edible fungi to lixiviation and to the cellulosic residue he gave the name "fongine." Various attempts were made to identify this material with true cellulose. In 1859 Frémy (40) showed that in many species of fungi the cellulosic material is not soluble in Schweitzer's reagent, which dissolves pure cellulose; he therefore gave to this material the name "metacellulose." As methods were developed for the identification of cellulose it became more and more apparent that this cellulosic material which makes up such a large part of the fungous tissue is not cellulose but a somewhat similar cellulosic complex. After over three-quarters of a century of research along this line it remained for Winterstein (127, 131) to show that this cellulosic complex contains nitrogen. His preparations from fungous tissue of various species, which he carefully freed from nucelin and albumen by treatment with caustic potash and Schulze's reagent, contained from 2.5 to 3.9 per cent nitrogen. On hydrolysis with 3 per cent sulfuric acid he (128) obtained d-glucose, acetic acid, and an undetermined nitrogenous organic substance. Later (129) he determined the combination of nitrogen in this substance by boiling with concentrated hydrochloric acid and obtained a chitosamine hydrochloride, $C_6H_{11}O_5NH_2 \cdot HCl$, which he called a glucosamine. He (130) finally isolated this chitinous material from *Agaricus campestris* and found its nitrogen content to be 6.24 per cent, a value agreeing fairly well with 6.01 per cent given for animal chitin, although in an earlier work (128) he reported nitrogen contents of this material of less than 1 per cent in some cases and with many determinations ranging around 3 per cent. In working with *Boletus edulis*, Reuter (88) found a nitrogenous residue which gave no protein reaction but proved to be a glucosamine or chitinous substance. He also found a carbohydrate like hemicellulose.

Chemists are prone to treat this chitinous material as a definite chemical compound and various formulas have been proposed for it. More recent workers (14, 59) have determined that when a purified product from crab shells is hydrolyzed, it yields nitrogen, acetic acid, and glucose in the ratio of 1:1:1. The latter authors have proposed that it is a polymer of four molecules of mono-acetyl-glucosamine, having a formula of $C_{32}H_{54}N_4O_{21}$, with the amino group joined to the glucose molecule on the one hand and to the acetyl group on the other. Whether this substance is always a definite chemical compound with one amino group to each glucose molecule or whether the nitrogen may

be more or less variable, being replaced in many cases by the -OH group of the glucose as it is in cellulose, is not definitely known. It would seem from the cellulosic nature of this substance that it may vary from the formula given above for chitin, which has a glucose-nitrogen ratio of 1:1, to that of cellulose which contains no nitrogen. The variable results of early workers and the variable nitrogen figures given by Winterstein point very strongly to the latter view.

Little is known regarding the amount of this cellulosic material. Unpublished work done at the Forest Products Laboratories, Madison, Wisconsin, indicates that about half of the dry weight of the mycelium is left after the process of chlorination, but nothing definite is known as to the constitution of this material. Tanret (102) reported 15 per cent of chitin in the mycelium of *Aspergillus niger;* Proskuriakow (84), 5.5 per cent in the fruiting bodies of *Agaricus campestris;* and Winterstein and Reuter (132) found 6 per cent of this material in the tissue of *Boletus edulis.* From the fruiting bodies of *Lycoperdon pyriforme,* Iwanoff (54) isolated an alcohol-insoluble material which yields glucosamine on hydrolysis. He (53) also reported viscosin from the same organism in amounts varying from 11 to 26 per cent. This material contains from 6.1 to 6.5 per cent of nitrogen and gives glucosamines on hydrolysis. He considered this material along with the chitosans as an intermediate product in the synthesis of chitin from glucose.

DECOMPOSITION AND NITRIFICATION OF FUNGOUS TISSUE

Since the mycelial tissue of soil fungi probably makes up a large part of the microbial materials of the soil, the decomposition of this material and especially the change of its nitrogen to the inorganic form is a very important process in relation to soil fertility. Since the saprophytic soil fungi are unable to fix free nitrogen they must obtain this element from the combined nitrogen of the soil, either from solution or by the hydrolysis of organic nitrogenous compounds. In the use of soil nitrogen the fungi have a distinct advantage as compared to the higher plants in that the fungi are able to assimilate their nitrogen before it reaches the nitrate stage, which most green plants are unable to do. The fungi absorb the mineral soil nitrogen, quickly converting it into organic form, and thus it is removed from the sphere of availability for higher plants.

Whether this nitrogen which has been stored up in the tissues of these organisms is again readily available for higher plants or whether it is more or less resistant and unavailable has been given little attention by research workers and very little is reported on the decomposition of fungous material. Starkey (100) used the evolution of carbon dioxide as an index of the decomposition of organic matter in the soil and found that on decomposition a fungous material containing 3.84 per cent of nitrogen and 44.3 per cent of carbon evolves carbon dioxide to a greater extent than alfalfa meal and almost equal to dried blood. The alfalfa meal in this case contained 2.45 per cent of nitrogen and 40.62 per cent of carbon, and the dried blood 9.61 per cent of nitrogen and

37.52 per cent of carbon. He reported 67 per cent of the carbon of the fungous material lost in 10 days. When nitrate nitrogen is added the decomposition is accelerated. Rege (87) found that when the mycelial tissue of a *Coprinus* sp., which contained 3.5 per cent of nitrogen, was placed in the soil at the rate of 55 p.p.m. of nitrogen, the yields of mustard were inferior to those where dried blood and ammonium sulfate containing equal quantities of nitrogen were used, and very little greater than the control. His figures are as follows:

Control	3.85 gm. mustard (dry)
Ammonium sulfate	7.40 gm. mustard (dry)
Dried blood	7.10 gm. mustard (dry)
Coprinus sp.	4.70 gm. mustard (dry)

This work indicates that the nitrogen in this fungous tissue does not readily respond to the process of nitrification and is therefore not easily made available for plant use, although this material contained 3.5 per cent of nitrogen, a figure rather high for a green manure. Falck (38) showed that the nitrogen in fungous tissue is not available for the use of flax, oats, or peas but that young pines are able to use this nitrogen, perhaps because of their association with mycorrhiza fungi. In his work, manure containing much energy material was present in the sand cultures, which may help to explain his results.

In contrast to this work, Barthel and Bengtsson (10) very recently report that the mycelium of *Aspergillus niger* is readily decomposed and nitrified in the soil. Bierema (12) reported that from 0 to 40 per cent of the nitrogen in the tissue of *Penicillium glaucum* and *Mucor racemosus* is changed to nitrate in two months. He thinks that the older the culture and the more spores present, the slower the nitrification. The process of sterilization seems to decrease the rate of nitrate accumulation.

No attempt is made to review all the literature dealing with fungi, but only that which applies particularly to the subject at hand. Lafar (65), Russell (89), Waksman (115), and Zellner (136) have each given good reviews of various phases of the literature on fungi.

EXPERIMENTAL WORK

SOURCES OF FUNGOUS MATERIAL

Collection of fungous tissue from natural habitats

Large quantities of fungous tissue were necessary for the decomposition studies and also for chemical analyses. Many species were used in order that conclusions might not be drawn from insufficient data.

Attention was first directed to the many forms of the higher fungi found growing in nature and commonly known as mushrooms. Collections were made during September, October, and November. These materials were all of the higher fungi, and the ones collected were mainly the gill forms. The tissue obtained was only the aerial portion, consisting of the stipe and the pileus

of the fruiting body. It was impossible to obtain the mycelial tissue or hyphae from the substrate in quantities sufficient for either chemical or nitrification studies.

The fruiting or aerial portions were gathered, and while still fresh were separated from all foreign matter and washed with distilled water. They were then divided into small parts, placed on trays with screen bottoms, and the temperature quickly raised to 60°C. for a short time to stop the action of enzymes. The temperature was then lowered to about 40°C. and the material was quickly dried by means of an air current. It was later dried at 65°C. for 48 hours and ground to pass a 40-mesh sieve. In two cases the stipes were separated from the pilei.

The following species were collected from their natural habitats and the figures give the approximate amounts of dry material obtained:

	gm.
1. *Secotium acuminatum* Mont.	500
2. *Marasmius oreades* (stipes)	33
3. *Marasmius oreades* (pilei)	75
4. *Lycoperdon pyriforme*	240
5. *Coprinus* sp. (stipes)	140
6. *Coprinus* sp. (pilei)	60
7. *Pholiota adiposa*	100
8. *Clitocybe multiceps*	310
9. *Fomes igniarius*	200
10. *Polyporus graveolens**	80

* Species is questionable.

These species are all members of the higher group of fungi and were collected during the rainy fall months. They were all found growing where there was a more or less abundant supply of cellulosic energy material in the substrate. The *Pholiota, Fomes,* and *Polyporus* species were collected from the dead stumps of trees. The other species were found growing either on the ground or on manure. The *Coprinus* sp. is one of the inky cap mushrooms and was collected from a manure heap. The *Marasmius oreades* is the fairy ring fungus which grows in more or less complete rings on the lawn. The rings range from 1 or 2 to 10 or 12 feet in diameter. The *Secotium acuminatum* was found growing on an earth-covered compost heap. The fruiting bodies were numerous and as large as three inches in diameter. The *Clitocybe multiceps* and *Lycoperdon pyriforme* were found growing on pieces of decaying wood in the surface soil. All of these species were produced on a substrate low in available nitrogen and high in cellulosic energy material.

Attempts were made to produce the higher forms on large artificial manure cultures. Large outdoor bins were packed with 1000 pounds of straw and treated with lime; lime and ammonium sulfate; lime, ammonium sulfate, and acid phosphate; and lime and legume hay. Large steel tanks were set up indoors and 100-pound quantities of chopped straw were treated in the same way. These artificial manure cultures produced a number of *Coprinus* sp. but not in sufficient quantities to be of any value.

Production of fungous tissue on synthetic liquid media

Production in 1926. Pure cultures of *Aspergillus oryzae* were first grown on a modification of Pfeffer's solution as described by Steinberg (101). This medium was further modified to give three culture solutions of widely different carbon-nitrogen ratios. The culture solutions were made up by adding water and varying amounts of ammonium nitrate to a stock solution.

Stock solution

Sugar (sucrose)	100 gm.
Mono-potassium phosphate	10 gm.
Magnesium sulfate	5 gm.
Ferrous sulfate	Trace
Zinc sulfate	Trace
Water	1000 cc.

Culture solutions

1. High nitrogen solution (Carbon-nitrogen ratio = 6:1)	
Stock solution	1000 cc.
Ammonium nitrate	20 gm.
Water	1000 cc.
2. Medium nitrogen solution (Carbon-nitrogen ratio = 30:1)	
Stock solution	1000 cc.
Ammonium nitrate	4 gm.
Water	1000 cc.
3. Low nitrogen solution (Carbon-nitrogen ratio = 150:1)	
Stock solution	1000 cc.
Ammonium nitrate	0.8 gm.
Water	1000 cc.

Fifty-cubic centimeter portions of these solutions were placed in 12-ounce prescription bottles and sterilized at 15 pounds pressure for 30 minutes. They were then seeded with spores of *Aspergillus oryzae* and incubated in a horizontal position for 14 days at which time the pads were harvested. After being drawn from the bottles, the pads were placed in cold water and washed for an hour in several changes of tap water and finally in two or three changes of distilled water to free them from as much water-soluble material as possible. No doubt there was some loss of organic material from the broken hyphae. The pads were then dried at room temperature for 12 hours and finally in an oven at 37°C. This drying did not remove all of the water, for from 3.34 to 6.25 per cent remained. The material was then ground to pass a 60-mesh sieve.

The differences in the character of growth on the three solutions were very marked. The high nitrogen pads produced practically no spores, the medium nitrogen cultures fruited rather well, whereas those growing on the low nitrogen medium fruited profusely and rather early. In each case at the end of 14 days all of the sugar in the culture solution was consumed.

Two species of the higher fungi, *Coprinus radians* and *Psilocybe atamatoides*,

were grown on a 6 per cent (by weight) malt extract medium made up from a malt extract having the following composition:

	per cent
Maltose	51.02
Dextrose	10.94
Albuminate	3.11
Nitrogen (Kjeldahl)	0.95
Glycerine	9.27
Free acid	0.43
Inorganic substance	1.16
Water	22.47

The mycelium was grown, washed, dried, and handled in much the same way as was that of the *Aspergillus oryzae.* This particular *Coprinus radians* will be designated as *Coprinus radians* (*1926*) to distinguish it from that later grown on a malt-sugar solution.

Production in 1927.—In later attempts to produce pure cultures of fungi on liquid media so that the entire mycelial structure could be obtained, it was found that the higher forms grew very poorly on any synthetic medium tried, and that best growth was secured with a malt solution.

The lower forms, however, grew well on synthetic media, but each species of organism has its own particular requirements in the solution medium. After many trials a modification of the peptone nutrient medium of Klotz (61) was used for the production of *Trichoderma lignorum* and *Aspergillus oryzae.*

Modified peptone nutrient medium

Sucrose	30 gm.
Mono-potassium phosphate	5 gm.
Ammonium nitrate	2 gm.
Peptone	5 gm.
Magnesium sulfate	0.2 gm.
Calcium sulfate	0.2 gm.
Ferrous sulfate	Trace
Sodium chloride	Trace
Phosphoric acid	to pH 5
Water	1000 cc.

The *Trichoderma lignorum* grown on this medium produced an average dry pad of about 590 mgm. per 50 cc. of solution. On this medium, growth was normal and very good, giving a thick pad with only light sporulation. On the contrary Pfeffer's solution used in earlier work produced a very light pad and sporulated profusely. The *Aspergillus oryzae* did not grow so well on this solution as on Pfeffer's. The average weight of dry pad was only about 400 mgm. per 50 cc. of solution. This particular tissue of *Aspergillus oryzae* is designated as *Aspergillus oryzae* (*1927*).

For the growth of the higher forms a malt-sugar solution was used. The

analysis of this malt extract has been given and the composition of the solution was as follows:

Malt extract	30 gm.
Sucrose	10 gm.
Water	1000 cc.

On this solution were grown *Coprinus radians* and a *Coprinus* sp. (isolated from manure). The former species produced nearly a normal growth with some normal fruiting bodies and gave a dry pad weight of about 350 mgm., whereas the latter grew very poorly and gave a dry pad weight of between 180 and 200 mgm. per 50 cc. of solution.

This study seemed to indicate that if the fungi were grown on liquid media, a special solution would have to be found for each organism, as no two seem to give good growth with the same medium. Each solution with each organism produces its own particular pH reaction.

After removal from the medium the mycelial tissue was first washed and then dried, as previously explained, and ground to pass a 40-mesh sieve. Dry tissue of the four organisms grown in pure culture was obtained in the following approximate amounts:

	gm.
Trichoderma lignorum	100
Aspergillus oryzae (1927)	75
Coprinus radians (1927)	35
Coprinus sp.	20

CHEMICAL METHODS—SEPARATIONS

Water-soluble and -insoluble fractions

To secure the water-soluble and water-insoluble fractions of the fungous tissue and other organic matter, they were shaken with 60 cc. of water per gram of material and allowed to stand at room temperature with frequent shakings for 24 hours. They were then decanted through a C. S. & S. 589 white ribbon filter and washed with 40 cc. of water per gram used. Since the amount of filterable material depends much upon the grade of filter and method of filtering, this procedure was used in all separations for culture work and for the analysis of the culture material.

Dialization

The dializable portion was obtained by placing 3 gm. of the dry fungous tissue in a collodion sack with 25 cc. of water. The sack was then closed and suspended in a cylinder containing 150 cc. of water, and gently rotated. The water was changed four times during a 24-hour period, and the total solution taken off contained the dializable portion.

The contents of the sack were then placed in a beaker and diluted to 150 cc. with water, and after standing at room temperature a few hours were filtered

by suction through filter pulp on a Büchner funnel. The filter pulp and residue were returned to the beaker and this process was repeated twice. The filtrate contained the non-dializable portion of the water-soluble material.

Alcohol- and alkali-soluble and -insoluble fractions

It was found impracticable to use aqueous solutions, especially of sodium hydroxide, where both the filtrate and the residue were to be saved. To obviate this difficulty a 60 per cent alcoholic solution was used, and this was found to filter rather easily through hardened filter paper (C. S. & S.), with suction, both with the alcohol alone and with 0.05 *N* sodium hydroxide. Three grams of the material was digested at 50°C. in 100 cc. of the solvent for about 6 to 12 hours, filtered, and washed once or twice. It was then returned to the beaker and again digested in this way twice more, the whole process covering a 24-hour period. The filtrates were placed together and concentrated to volume.

The residue from the 60 per cent alcohol was then treated in the same way with 0.05 *N* sodium hydroxide in 60 per cent alcohol, giving in all three fractions, the alcohol-soluble, the alkali-soluble, and the residue, which were used for analysis.

ANALYTICAL METHODS

Total nitrogen. A modification of the Kjeldahl-Gunning method for total nitrogen was used in which mercuric oxide was the catalyst and sodium thiosulfate introduced before distillation for the precipitation of the mercury.

Ammonia nitrogen. Determinations were made by Harper's (43) method of extracting the wet soil with 10 per cent potassium chloride solution and subsequent distillation with heavy magnesium oxide. The ammonia was absorbed in an excess of standard acid and after the distillate had been boiled and cooled the excess acid was titrated to neutrality with standard alkali, using methyl red as the indicator.

Nitrate nitrogen. Harper's (44) modified phenol-di-sulfonic acid method was used with slight changes. The soils were dried at 65°C. for 24 hours to eliminate the water factor; alum and a saturated solution of calcium hydroxide were used to secure flocculation.

Amino nitrogen. Van Slyke's (104, 105) method was used as outlined by Hawk. The apparatus used was equipped with a micro-pipette.

Urea. A modification of Marshall's (72) clinical method for urea was used. Methyl red was substituted for methyl orange, the solution made slightly acid with standard acid and after boiling, the excess was titrated with standard sodium hydroxide.

Total carbon. Carbon was determined by the wet combustion method as given by the Association of Official Agricultural Chemists (7). The apparatus used was a very simple modification of that used by White and Holben (122) in their perfected method. This apparatus will be described in another paper.

The carbon dioxide was absorbed in 0.5 *N* sodium hydroxide, precipitated as the carbonate with neutral 2 *N* barium chloride and the excess sodium hydroxide titrated with 0.5 *N* hydrochloric acid, using phenolphthalein as the indicator.

Carbon dioxide carbon. The carbon dioxide was absorbed and estimated as outlined for total carbon. Continuous aspiration over the surface of the soil was employed.

ANALYSIS OF FUNGOUS TISSUE AND OTHER MATERIALS

Materials grown in 1926

The carbon and nitrogen contents of the fungous mycelia produced in 1926 compare favorably with those given in table 1. The analyses of mycelium of

TABLE 2

Composition of pure cultures of Aspergillus oryzae (1926), Psilocybe atamatoides and Coprinus radians (1926)

Air dry material

	ASPERGILLUS ORYZAE (1926)			PSILOCYBE ATAMATOIDES	COPRINUS RADIANS (1926)
	High*	Medium*	Low*		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Moisture	6.25	4.25	3.34	0.71	1.11
Ash	7.05	7.19	9.25	3.39	4.97
Total carbon	40.48	41.10	39.55	....	....
Total nitrogen	6.42	3.83	1.89	2.26	3.21
Water-soluble nitrogen	3.46	1.70	0.89	1.07	1.65
Average weight of pad, mgm.	672	653	290	....	....
Carbon-nitrogen ratio of the tissue	6.3	10.7	20.9	....	....

* Refers to the nitrogen content of the culture medium. The ratio of the carbon to nitrogen in the high nitrogen medium was 6 to 1, in the medium nitrogen medium, 30 to 1, and in the low nitrogen medium 150 to 1.

Aspergillus oryzae grown on Pfeffer's solution and of *Coprinus radians* and *Psilocybe atamatoides* grown on a 6 per cent malt extract solution are given in table 2.

These data indicate three points that are borne out in subsequent work; (*a*) That the carbon content of the tissue is approximately constant, (*b*) that the nitrogen content varies inversely, and the ratio of carbon to nitrogen in the tissue directly, with the carbon-nitrogen ratio of the medium, and (*c*) that roughly half of the nitrogen in the dry fungous tissue is soluble in water.

The relation of the nitrogen in the substrate to that in the fungous tissue is significant. Vorbrodt (107) found that on a medium containing 1 per cent of ammonium nitrate, mycelium of *Aspergillus niger* containing 7 per cent of nitrogen is produced. But when the ammonium nitrate in the medium is

reduced to 0.05 per cent the mycelium contains only 2 per cent of nitrogen. In table 2 the high nitrogen medium contained 1 per cent and the low nitrogen medium 0.04 per cent of ammonium nitrate. These have produced mycelial tissue of *Aspergillus oryzae* with approximately the same nitrogen contents as the tissues of *Aspergillus niger* reported by Vorbrodt. These data show that where the ratio of carbon to nitrogen is between 6 and 30 to 1, the weight of the tissue produced on the same amount of medium is approximately the same but more nitrogen is assimilated by the organisms from the high nitrogen medium. As the carbon-nitrogen ratio of the medium is raised above 30 to 1 the weight of the mycelium decreases much faster than the nitrogen in the tissue, and a minimum nitrogen content of about 2 per cent is maintained. There is little drop in the weight of the mycelial tissue until its ratio of carbon to nitrogen reaches approximately 10 or 12 to 1. In this respect the fungi are much like the higher plants, as they are able to adjust their growth and nitrogen content to the supply of available nitrogen.

The nitrogen of the dry mycelial tissue is approximately 50 per cent soluble in water. With the *Aspergillus oryzae* there is a tendency for the tissue of highest nitrogen content to have the greatest solubility. Vorbrodt found the same to be true of *Aspergillus niger.*

Materials for carbon evolution and plant culture studies

Tables 3, 4, and 5 give the percentages of carbon and nitrogen in all of the materials used in the experimental work on the evolution of carbon dioxide and nitrate accumulation in soils and also in the plant culture work. Here again it will be noted that the carbon content of the materials is rather constant, with few exceptions falling between 41 and 44 per cent. As with the *Aspergillus oryzae* in table 2, the nitrogen content and the ratio of carbon to nitrogen vary greatly, depending on two factors, the carbon-nitrogen or rather the energy-nitrogen ratio of the substrate and the species of the organism. This variation of nitrogen content is not only true in the forms grown on synthetic media but is also true of the forms collected from their natural habitats. It is remarkable that the puffball, *Lycoperdon pyriforme,* growing on a piece of rotting wood in a very poor soil, should have a nitrogen content of over 5 per cent. The same is true of *Marasmius oreades,* which, growing in competition with lawn grass, has a nitrogen content of about 7 per cent.

As compared with plant material, most of the fungous tissue is high in nitrogen even though the tissue is produced on substrate of low nitrogen content. Although the pilei of the higher fungi contain more nitrogen than the stipes, the latter are higher in that element than ordinary plant tissue. The high nitrogen content of the mycelial tissue of the fungi indicates a rapid assimilation of nitrogen and this together with the fact that the fungi are able to use their nitrogen in the ammonia form makes it practically impossible for green plants to assimilate soil nitrogen when energy material and fungi are present in the soil.

The water solubility of the fungous nitrogen varies greatly with the species and with the substrate upon which the tissue is produced. The solubility of the nitrogen in water varies from a little less than half, in the *Aspergillus oryzae* (1926 medium nitrogen), to about five-sixths, in the *Clitocybe multiceps*. With cottonseed meal only a small part is water-soluble and with alfalfa hay approximately two-fifths is dissolved in water.

TABLE 3

Carbon and nitrogen content of organic materials and fungous tissues

Air-dry material

MATERIAL	CARBON	NITROGEN
	per cent	*per cent*
Ammonium sulfate	....	21.05
Blood meal	46.8	14.74
Cottonseed meal	44.6	6.64
Alfalfa hay	43.7	2.58
Timothy hay	44.5	1.19
Straw (*mixed grain*)	44.4	1.04
Saccharomyces cerevisiae	43.8	9.53
Trichoderma lignorum	44.4	5.13
Aspergillus oryzae (1927)	41.9	4.58
Aspergillus oryzae (1926 Med. N)	41.1	3.83
Coprinus radians (1927)	41.2	2.27

TABLE 4

Carbon and nitrogen content of higher fungi collected from their natural habitats

Air-dry material

MATERIAL	CARBON	NITROGEN
	per cent	*per cent*
Secotium acuminatum	41.2	7.71
Marasmius oreades (pilei)	42.4	7.10
Marasmius oreades (stipes)	41.8	6.37
Coprinus sp. (pilei)	42.2	6.42
Lycoperdon pyriforme	42.5	5.04
Coprinus sp. (stipes)	38.8	4.00
Pholiota adiposa	41.7	3.84
Clitocybe multiceps	40.6	3.74
Fomes igniarius	44.3	1.71
Polyporus graveolens	41.7	1.56

Distribution of carbon and nitrogen in fungous tissue

The tissues of *Lycoperdon pyriforme* and *Trichoderma lignorum*, which represent different forms produced under widely different conditions, were used throughout this work. The former is one of the higher forms found growing in nature on rotting wood and the latter is one of the molds or lower forms produced on a synthetic liquid culture medium.

These tissues were separated into fractions by two methods; first, by water solubility, in which the dializable, the water-soluble non-dializable, and the water-insoluble fractions were obtained, and second, by alcohol and alkali solubility, in which the alcohol- and alkali-soluble and the alkali-insoluble fractions were obtained. Tables 6 and 7 give a summary of this work.

TABLE 5

Carbon and nitrogen content of the water-soluble and water-insoluble fractions of various materials

Air-dry material

MATERIAL	CARBON	NITROGEN
	per cent	*per cent*
Cottonseed meal, water-soluble	7.1	0.74
Cottonseed meal, water-insoluble	37.6	5.90
Alfalfa hay, water-soluble	12.9	1.05
Alfalfa, hay, water-insoluble	30.8	1.53
Lycoperdon pyriforme, water-soluble	17.2	2.83
Lycoperdon pyriforme, water-insoluble	25.3	2.21
Lycoperdon pyriforme, 0.3 per cent NaOH insoluble	18.1	1.72
Trichoderma lignorum, water-soluble	19.7	3.48
Trichoderma lignorum, water-insoluble	24.7	1.65
Clitocybe multiceps, water-soluble	21.4	3.15
Clitocybe multiceps, water-insoluble	19.2	0.59
Clitocybe multiceps, 0.3 per cent NaOH insoluble	15.1	0.60
Aspergillus oryzae (1926 Med. N), water-soluble	5.7	1.73
Aspergillus oryzae (1926 Med. N), water-insoluble	35.4	2.10

In both of the species reported, over half of the weight of the material and from 50 to 70 per cent of the nitrogen is soluble in water, which is in accord with the work of Vorbrodt (107) with *Aspergillus niger*. Of this water-soluble nitrogen less than 10 per cent is non-dializable and approximately half is in the free amino form. These facts indicate that the nitrogen of the water-soluble fraction is made up of very simple proteins and amino acids. The higher water solubility is in the less mature tissue of *Trichoderma* grown on liquid culture medium, and indicates that age and amount of available nitrogen affect the solubility of the nitrogen in the tissue.

The alcohol-soluble portion is not so large as the water-soluble fraction but has about the same proportion of amino nitrogen. The alkali-soluble fraction makes up from 45 to 60 per cent of the total nitrogen and is less simple than the water-soluble fraction, being only 10 to 15 per cent amino nitrogen. This

fraction shows more complex proteinaceous materials which are not water-soluble. From 16 to 18 per cent of the nitrogen in the tissue is insoluble in

TABLE 6

Distribution of carbon and nitrogen in the tissue of Lycoperdon pyriforme

Calculated in 1 gm. of air-dry material

FRACTION	WEIGHT OF MATERIAL	CARBON	TOTAL NITROGEN		AMINO-NITROGEN	
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Total (original sample)†	1,000	425	50.4			
Water-soluble fraction						
Dializable	430	162	24.3	48.2	10.25	42.2
Non-dializable	93.3	31.5	2.62	5.2	1.69	64.5
Water-insoluble fraction*	476.7	231.5	23.5	46.6		
Alcohol-soluble fraction‡	375	140.6	18.0	35.7	9.14	50.8
Alkali-soluble fraction§	148*	92.6	23.12	45.9	3.28	14.2
Alcohol- and alkali-insoluble fraction	451	175.5	9.25	18.3		
Loss		16.3	0.03	0.1		

* By difference.

† Moisture 2.61 per cent.

‡ 60 per cent alcohol.

§ 0.05N sodium hydroxide in 60 per cent alcohol after the alcohol-soluble fraction was removed.

TABLE 7

Distribution of carbon and nitrogen in the tissue of Trichoderma lignorum

Calculated in 1 gm. of air-dry material

FRACTION	WEIGHT OF MATERIAL	CARBON	TOTAL NITROGEN		AMINO-NITROGEN	
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Total (original sample)	1,000	444	51.3			
Water-soluble fraction:						
Dializable	416.4	177.6	33.17	64.6	15.65	47.1
Non-dializable	97.1	38.0	2.59	5.05	1.31	57.5
Water-insoluble fraction*	486.5	228.4	15.54	30.35		
Alcohol-soluble fraction†	237.6	105	9.2	18.0	5.68	61.7
Alkali-soluble fraction‡		124.2	32.2	62.8	2.96	9.2
Alcohol- and alkali-insoluble fraction	344.8	137.9	8.36	16.3		
Loss		76.9	1.54	2.9		

* By difference.

† 60 per cent alcohol.

‡ 0.05N sodium hydroxide in 60 per cent alcohol after the alcohol-soluble fraction was removed.

0.05 N sodium hydroxide. This may be reduced somewhat by using stronger alkali, but usually from 12 to 15 per cent of the fungous nitrogen remains in

the alkali-insoluble residue. The form of this nitrogen is not known, but it must be of a more or less complex protein nature with perhaps some chitinous forms. This residual material is no doubt one of the contributing factors mentioned by Waksman (114) in the composition of soil humus.

Although Iwanoff (51, 52, 55, 56, 57) has reported as much as 8.5 per cent of urea in *Psalliota campestris*, and 4.3 per cent in *Lycoperdon pyriforme*, none could be found in either the *Lycoperdon pyriforme* or the *Trichoderma lignorum* used in this work.

On the whole it appears from the data given and from the work of other investigators, that much of the nitrogen in fungous tissue is readily soluble in water and of a simple nature. Although the insoluble residue may contain some chitinous nitrogen, the amount is small and cannot possibly be of any considerable importance in the mineralization of fungous nitrogen.

LABORATORY AND GREENHOUSE WORK ON THE AVAILABILITY OF FUNGOUS NITROGEN

Nitrification studies

It has already been shown that the fungi and particularly those forms living in the soil use much inorganic nitrogen and convert it into organic forms in their tissue. The amount of nitrogen in fungous material is variable and relatively high. It is approximately half soluble in water and largely of simple structure. In order to study the return of this nitrogen to the inorganic form and the factors which govern this mineralization process, three sets of experiments were carried out: first, nitrification studies on fungous tissue of the same species with varying nitrogen contents; second, nitrification studies of various species with a study of the carbon relations; and third, a study of the assimilation of the nitrogen from fungous tissue by higher plants. In the first part, the tissues of *Aspergillus oryzae* (1926), *Coprinus radians* (1926), and *Psilocybe atamatoides* were used.

The air-dried material as well as the living pads as they were removed from the wash water, were placed in 1200 gm. portions of sifted and well-mixed Miami silt loam at the rate of 2 tons (2.4 gm. per pot) of dry material per acre. As this soil was acid (pH 5.8), precipitated calcium carbonate was added at the same rate. The moisture was made up to 20 per cent and held constant. The work was carried out in half-gallon earthenware pots with incubation at 23°C.

The tissue of *Aspergillus oryzae* used in this work was added to the soil in six different forms as follows:

1. Living mycelium; the living tissue on being removed from the wash water was divided into small pieces and placed directly into the moist soil.
2. Living mycelium dried after 20 days; same as no. 1 but after being in the soil for 20 days the entire soil mass was spread out and air-dried for 48 hours. It was then returned to the pot and made up to its original moisture content.
3. Sterilized mycelium; the dry tissue was sterilized at 15 pounds steam pressure for 45 minutes before being placed in the soil.

4. Dry mycelium; the tissue was dried at 65°C. for 48 hours before being placed in the soil.
5. Water-soluble fraction; the water-soluble fraction of no. 4.
6. Water-insoluble fraction; the water-insoluble fraction of no. 4.

In the Aspergillus series there were some variations in the amounts of nitrogen and dry matter where the living materials were added. Whole pads were used

TABLE 8

Number of bacteria per gram of soil according to plate count

TREATMENT PER POT	AFTER 30 DAYS	AFTER 40 DAYS
	millions	*millions*
Controls	16.3	14.4
Controls, dried after 20 days*	8.0	18.8
Aspergillus oryzae, 6.42 per cent N		
Living mycelium, 4 pads	61.6	92.6
Dried after 20 days	.....	92.6
Sterilized mycelium, 2.4 gm	126.0	265.0
Dry mycelium, 2.4 gm	106.0	184.3
Water-soluble fraction	49.0	34.3
Water-insoluble fraction	79.0	105.3
Aspergillus oryzae, 3.38 per cent N		
Living mycelium, 4 pads	59.0	100.6
Dried after 20 days	.....	157.6
Sterilized mycelium, 2.4 gm	132.0	271.0
Dry mycelium, 2.4 gm	101.0	240.3
Water-soluble fraction	27.0	42.3
Water-insoluble fraction	86.3	202.6
Aspergillus oryzae, 1.89 per cent N		
Living mycelium, 8 pads	67.0	109.6
Dried after 20 days	.....	110.6
Sterilized mycelium, 2.4 gm	117.0	176.0
Dry mycelium, 2.4 gm	106.0	245.6
Water-soluble fraction	23.0	41.0
Water-insoluble fraction	92.0	95.0
Wheat Straw		
Finely ground, 2.4 gm	31.0	21.7

* After 20 days, the contents of the pots were removed, air dried for 48 hours, and returned.

and their dry weights were not exactly the same as those of the dry materials. The amounts of nitrogen added are given in table 9.

McLennan (71) has very recently shown that the air drying of fungous tissue kills the vegetative hyphae but that this treatment has no effect upon the spores. When a soil containing living fungous hyphae is air dried, as in the case of no. 2 above, it is reasonable to expect that this tissue will be killed.

TABLE 9

The accumulation of nitrate nitrogen from decomposing fungous tissue in soil

Calculated in 100 gm. of soil

TREATMENT PER POT	NITROGEN ADDED	NITRATE NITROGEN RECOVERED			PERCENTAGE OF NITROGEN RECOVERED		
		20 days	40 days	80 days	20 days	40 days	80 days
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*
Aspergillus oryzae, 6.42 per cent N							
Living mycelium, 4 pads	14.83	8.4	9.5	11.2	56.5	63.8	75.3
Dried after 20 days	14.83	8.5	9.6	10.9	57.3	64.7	73.4
Sterilized mycelium, 2.4 gm.	12.84	4.54	5.3	6.0	35.4	41.2	46.7
Dry mycelium, 2.4 gm.	12.84	6.2	6.8	7.6	48.3	52.9	59.4
Water-soluble fraction	6.92	4.96	5.0	4.93	71.6	72.2	71.3
Water-insoluble fraction	5.92	0.96	1.44	1.8	16.2	24.3	30.2
Aspergillus oryzae, 3.38 per cent N							
Living mycelium, 4 pads	9.14	2.31	3.62	4.17	25.2	39.6	45.8
Dried after 20 days	9.14	2.16	3.00	3.46	23.6	32.8	37.8
Sterilized mycelium, 2.4 gm.	7.66	0.61	1.12	1.50	6.9	14.7	19.4
Dry mycelium, 2.4 gm.	7.66	1.01	1.50	2.50	13.2	19.6	32.7
Water-soluble fraction	3.40	1.96	2.00	2.15	57.7	58.9	63.3
Water-insoluble fraction	4.26	−0.34	0.37	0.66	....	8.8	15.5
Aspergillus oryzae, 1.89 per cent N							
Living mycelium, 8 pads	3.73	−0.62	0.67	1.00	....	17.9	26.7
Dried after 20 days	3.73	−0.67	0.37	0.60	....	9.8	16.1
Sterilized mycelium, 2.4 gm.	3.78	−1.84	−0.77	0.15	....	....	4.0
Dry mycelium, 2.4 gm.	3.78	−1.86	−0.97	−0.25	....	....	....
Water-soluble fraction	1.78	0.81	0.87	1.00	45.4	49.2	56.3
Water-insoluble fraction	2.00	−2.02	−1.43	−0.67	....	....	....
Wheat straw							
Finely ground, 2.4 gm.	0.76	−2.43	−1.92	−1.35	....	....	....
Psilocybe atamatoides							
Dry mycelium, 2.4 gm.	4.52	−1.03	.....	.....	....	....	....
Water-soluble fraction	2.14	0.50	.....	.....	23.3	....	....
Water-insoluble fraction	2.37	−1.22	.....	.....	....	....	....
Coprinus radians (1926)							
Dry mycelium, 2.4 gm.	6.42	1.09	.....	.....	17.0	....	....
Water-soluble fraction	3.30	1.79	.....	.....	54.2	....	....
Water-insoluble fraction	3.12	0.11	.....	.....	3.85	....	....

— means below the control.

Its decomposition will then be the same as that for dead tissue. The data given in tables 8 and 9 and also shown graphically in figures 1 and 2 show the effects of these various treatments upon the nitrification of fungous tissue.

Number of bacteria and amount of fungous growth

After 30 and 40 days, bacterial counts of these soils were made on 0.1 per cent nutrose (sodium caseinate) agar. Table 8 shows the number of bacteria per gram of soil for the various treatments including wheat straw at the rate of 2 tons per acre.

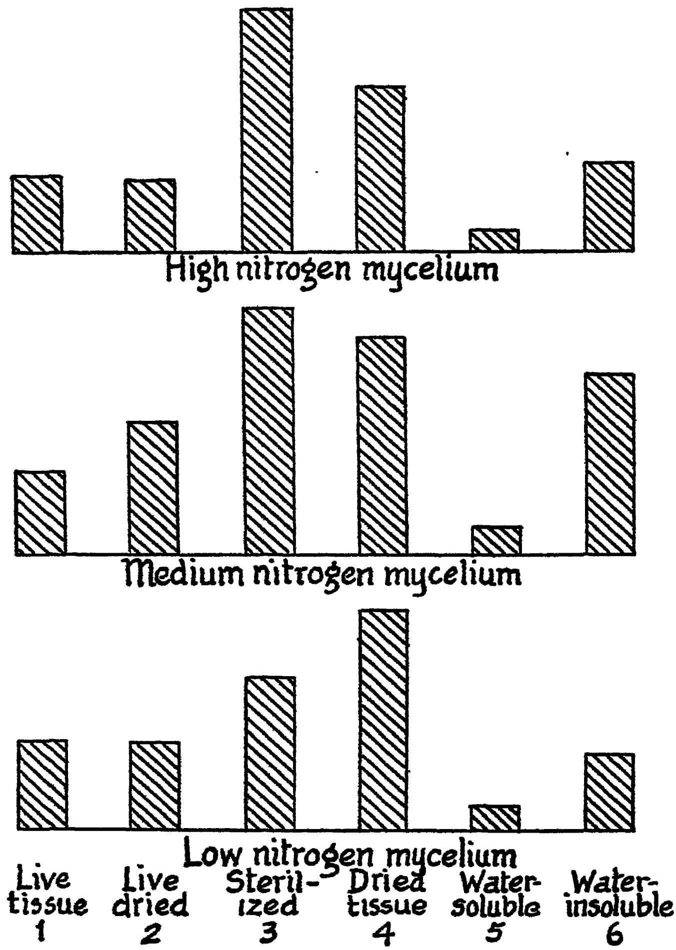


FIG. 1. RELATIVE BACTERIAL COUNTS FROM THE TISSUE OF ASPERGILLUS ORYZAE AFTER 40 DAYS IN THE SOIL

1. Fungous tissue incorporated in the soil in the living state.
2. Same as no. 1, but at the end of 20 days the entire soil mass was spread out and air-dried for 48 hours.
3. Sterilized at 15 pounds steam pressure for 45 minutes before being placed in the soil.
4. Fungous tissue dried at 65°C. before being placed in the soil.
5. Water-soluble fraction of no. 4.
6. Water-insoluble fraction of no. 4.

The number of bacteria ranged from 14.4 millions per gram of soil in the control to as high as 271 millions per gram where the mycelial tissue was sterilized with steam before its addition to the soil. The data in table 8 indicate that the number of bacteria are in direct relation to the amount of energy material suitable for their growth. Waksman and Starkey (121) have shown that glucose increases the number of bacteria in the soil and that cellulosic materials increase the amount of fungous tissue produced. The lowest bacterial count for any treatment was that for wheat straw, a substance low in soluble material but high in cellulosic energy material. In this case however, there was a very heavy mold growth in the soil. The dry mycelial tissue, on the other hand, gave high counts because of its high content of water-soluble energy material in the forms of the simpler carbohydrates, proteins, and amino acids. When the tissue was sterilized, the count was still higher because of the hydrolysis of some of the more complex substances. Living tissue gave a lower count than the dead tissue, there being less immediately available energy substance. The number of bacteria produced from the dry mycelium is approximately equal to the sum of those produced from its fractions. Figure 1 shows the relative number of bacteria produced from the various tissues of *Aspergillus oryzae.*

The amount of fungous material produced for each treatment could not be measured, but observation indicated heavy mold growth on all treatments where cellulosic or insoluble materials were added. The controls and the water-soluble fractions gave no noticeable fungous growth. The live tissue continued its growth from the energy in its own system but the dead tissue was immediately attacked by the saprophytic soil fungi and the entire soil was filled with their hyphae. There is no particular correlation between the number of bacteria and the amount of fungous hyphae produced, each developing independently of the other but depending upon the amount and kind of energy materials present. This is directly in line with the work of Fleming (39) who found that the development of bacteria and fungi in a soil depends upon the presence of organic matter, and that there is no evidence of a depressing effect of one upon the other, but that the relative abundance of the two groups depends upon their relative ability to utilize the energy materials present.

Accumulation of nitrate nitrogen

After intervals of 20, 40, and 80 days the accumulation of nitrate nitrogen from fungous tissue was determined. These data together with the percentages of the availability of the fungous nitrogen are given in table 9.

It is apparent that the more nitrogen added per unit of material the higher the availability of the nitrogen. This results from the fact that a given amount of energy material requires a certain amount of nitrogen for its decomposition, and any nitrogen in excess of this amount is liberated as nitrate. The figures show that from 200 mgm. of the dry tissue of *Aspergillus oryzae* in 100 gm. of

soil, from 4 to 5.2 mgm. of nitrogen remains unliberated as nitrate in 80 days. The amount of nitrogen not liberated in this way is fairly constant for a given amount of tissue during any period regardless of its nitrogen content, and roughly represents the amount of nitrogen used by soil microorganisms. If then the material contains less than this amount of nitrogen, none is liberated,

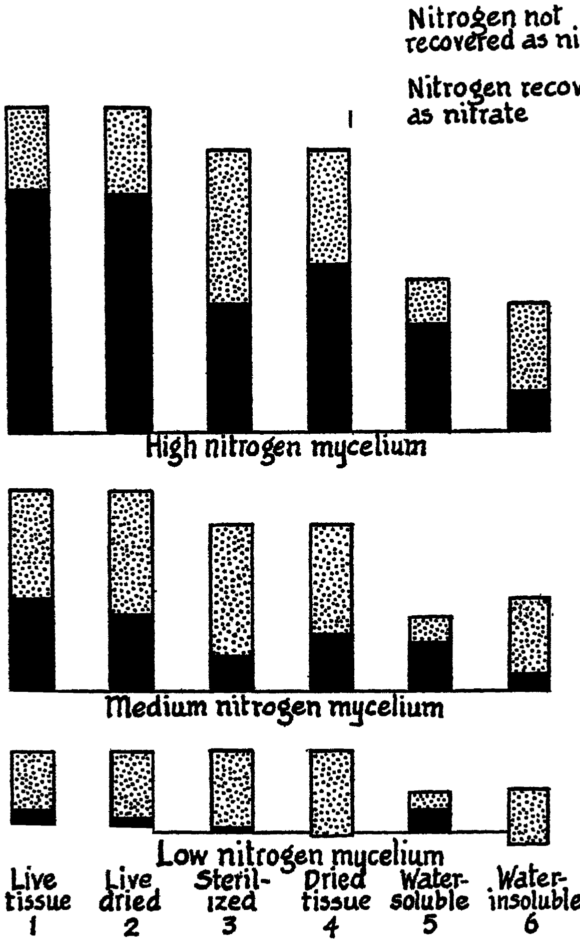


FIG. 2. RELATIVE AVAILABILITY OF THE NITROGEN IN THE TISSUE OF ASPERGILLUS ORYZAE AFTER 80 DAYS IN THE SOIL

1. Fungous tissue incorporated in the soil in the living state.
2. Same as no. 1, but at the end of 20 days the entire soil mass was spread out and air-dried for 48 hours.
3. Sterilized at 15 pounds steam pressure for 45 minutes before being placed in the soil.
4. Fungous tissue dried at 65°C. before being placed in the soil.
5. Water-soluble fraction of no. 4.
6. Water-insoluble fraction of no. 4.

but if it contains more than this amount, nitrate nitrogen is formed to the extent of the excess.

The nitrogen in living fungous tissue placed in the soil in the absence of other energy material is even more available than that in dead tissue. Drying at the end of 20 days tends to lessen the amount of nitrogen made available as nitrate. Observations indicate that in the living state fungous growth is continued and the energy for that growth in the absence of other energy material is derived from its own tissue through its autolysis. Dox (29, 30) and Dox and Maynard (32) found that when the energy material is exhausted from a culture medium, the organisms begin to autolize, using the energy from their own systems and liberating part of the nitrogen which they contain. The dry and sterilized tissues, both of which are dead, have a lower nitrogen availability than the living tissue. This is perhaps due to the heavy growth of saprophytic fungi on these tissues, although this is not true of the living material.

The water-soluble material from the fungous tissue has a higher nitrogen availability than either the water-insoluble or the total and its availability reaches its maximum in 20 days with no increase up to 80 days. This rapid nitrification is in accord with the findings of Whiting and Richmond (124), working with the parts of the sweet clover plant, that the roots, which contain large amounts of water-soluble material, nitrify much more rapidly at first than the leaves or stems. The water-soluble fungous nitrogen gives a high availability due largely to low cellulosic energy material and low fungous growth. The algebraic sum of the nitrogen not liberated from the water-soluble and water-insoluble fractions for any given period approximate that not liberated from an equal amount of the total tissue, and in every case the high energy residue has the low availability which is most marked in the low nitrogen tissue.

There is very little correlation of the availability of nitrogen with the number of bacteria but rather with the presence of energy material and more particularly with fungous growth. When the energy material is high and of such a nature as to produce a heavy fungous growth, the nitrogen availability is low. This is true in every case under observation in this work. It seems logical to believe that cellulosic energy materials in the soil cause the growth of saprophytic fungi and that these organisms use nitrogen in proportion to their growth or in proportion to the energy materials used, liberating only the nitrogen in excess of that necessary to balance this energy factor.

Figure 2 shows graphically the availability of the nitrogen in the tissue of *Aspergillus oryzae* and also that part of the nitrogen held insoluble after 80 days in the soil because of the energy factor.

EVOLUTION OF CARBON DIOXIDE AND ACCUMULATION OF NITRATE NITROGEN

It has already been shown that the ratio of carbon to nitrogen in the tissue is an important factor in the mineralization of fungous nitrogen, but there are

indications that it is not the only factor operating in this process. In order to study the relation of carbon to the formation of nitrates from fungous tissue and other sources of organic nitrogen, a second series of nitrification tests were carried out in flasks in the following manner: 600-gm. portions (dry weight) of a sandy mixture, made up of 25 per cent Miami silt loam and 75 per cent sand, were placed in a 750-cc. Erlenmeyer flask. The fungous tissues and other substances in amounts equivalent to 60 mgm. of nitrogen, or the water-soluble or -insoluble fraction thereof, were mixed with the soil. The moisture

TABLE 10

Ammonia and nitrate nitrogen from the decomposition of fungous tissue and other materials after 10 days' incubation

100 p.p.m. of nitrogen added

	AMMONIA NITROGEN	NITRATE NITROGEN
	p.p.m.	*p.p.m.*
Control	8.7	6.1
Ammonium sulfate	11.4	96.6
Blood meal	10.9	63.4
Cottonseed meal	9.8	47.0
Alfalfa hay	11.4	10.1
Timothy hay	9.3	Trace
Straw	8.7	Trace
Saccharomyces cerevisiae	11.4	66.2
Secotium acuminatum Mont.	10.9	40.2
Marasmius oreades (stipes)	9.2	42.0
Marasmius oreades (pilei)	9.8	50.0
Lycoperdon pyriforme	9.8	34.0
Trichoderma lignorum	9.3	42.8
Coprinus sp. (stipes)	9.8	33.0
Coprinus sp. (pilei)	9.8	33.0
Pholiota adiposa	8.7	None
Clitocybe multiceps	9.3	24.1
Fomes igniarius	10.9	10.4
Polyphorus graveolens	9.8	Trace

content was adjusted to 9 per cent (by weight) which was the optimum for this sand and soil mixture. In addition to this amount of moisture, 2 gm. of water was added for each gram of the organic material. The soil mixture gave a slightly alkaline reaction. The flasks were connected with a carbon dioxide absorption apparatus, which will be described in a later paper. The carbon dioxide evolved was determined at intervals of 12 to 72 hours. The aspiration was continuous over the soil except while the titrations were being made. All soil cultures were incubated at room temperature.

The amounts of carbon evolved as carbon dioxide were determined for a

period of 26 days as were also the amounts of nitrate nitrogen accumulated at that time. Ammonia and nitrate determinations were also made on duplicate treatments after a 10-day period. These data are given in table 10.

TABLE 11

Production of carbon dioxide and accumulation of nitrate nitrogen from the decomposition of fungous tissue and other organic materials

10 mg. nitrogen added to 100 gm. soil

MATERIAL	AMOUNT ADDED		NITRATE NITROGEN AFTER 26 DAYS	NITROGEN NITRIFIED	CARBON EVOLVED AS CARBON DIOXIDE		CARBON NITROGEN RATIO OF MATERIAL
	Dry weight	Carbon			Total	Last 72 hours	
	mgm.	*mgm.*	*mgm.**	*per cent*	*mgm.**	*mgm.*†	
Organic fertilizers and green manures							
Ammonium sulfate	47.5	.....	9.35	93.5	5.9	0.05	....
Blood meal	67.8	31.7	6.75	67.5	19.7	0.50	3.2
Cottonseed meal	150.5	67.2	4.95	49.5	34.8	0.55	6.7
Alfalfa hay	387.5	169.3	1.65	16.5	94.5	2.02	16.9
Timothy hay	840.3	373.8	−1.05	None	126.7	5.60	37.4
Straw	961.5	427.0	−1.05	None	113.2	5.47	42.7
Fungous tissue							
Saccharomyces cerevisiae	104.8	46.0	5.95	59.5	31.5	0.17	4.6
Secotium acuminatum	129.6	53.5	3.62	36.2	30.1	0.45	5.3
Marasmius oreades (pilei)	140.8	59.6	4.25	42.5	38.3	0.47	6.0
Marasmius oreades (stipes)	157.0	65.6	3.85	38.5	41.7	0.55	6.6
Coprinus sp. (pilei)	155.8	65.6	3.05	30.5	29.4	0.55	6.6
Lycoperdon pyriforme	198.3	84.3	3.25	32.5	49.8	0.70	8.4
Trichoderma lignorum	195.0	86.5	3.95	39.5	57.1	0.72	8.6
Aspergillus oryzae (1927)	216.6	91.5	4.2	42.0	59.2	0.95	9.2
Coprinus sp. (stipes)	250.0	97.0	3.35	33.5	57.6	0.77	9.7
Aspergillus oryzae (1926, med. N)	261.2	107.3	3.55	35.5	68.4	0.90	10.7
Clitocybe multiceps	267.3	108.5	2.75	27.5	64.8	0.90	10.9
Pholiota adiposa	260.3	108.6	−0.40	None	65.7	1.75	10.9
Coprinus radians	440.5	181.5	0.55	5.5	69.4	2.05	18.2
Fomes igniarius	584.8	259.0	0.35	3.5	15.1	1.62	25.9
Polyporus graveolens	641.0	267.3	−0.93	None	35.9	5.12	26.7

— means less than the control.

* Total minus that of the control.

† Carbon evolved as carbon dioxide last 72-hour period.

Tables 11 and 12 show the evolution of carbon as carbon dioxide and the accumulation of nitrate nitrogen during a period of 26 days. These tables also show the amounts of dry matter, carbon, and nitrogen incorporated in 100 gm. of soil.

AMMONIA AND NITRATE ACCUMULATION

The nitrogen in the form of ammonia at the end of 10 days was much the same for all treatments, ranging from 9 to 11 p.p.m., which is near the minimum usually found in a soil. This low ammonia content indicates that in these cultures the use of nitrogen by soil microorganisms and the process of nitrate

TABLE 12

Production of carbon dioxide and accumulation of nitrate nitrogen from the decomposition of the water-soluble and -insoluble fractions of various materials

Solubility fractions in 100 gm. of soil

MATERIAL	AMOUNT ADDED		NITRATE NITROGEN AFTER 26 DAYS	NITROGEN NITRIFIED	CARBON EVOLVED AS CARBON DIOXIDE		CARBON-NITROGEN RATIO OF MATERIAL
	Carbon	Nitrogen			Total	Last 72 hours	
	mgm.	*mgm.*	*mgm.**	*per cent*	*mgm.**	*mgm.*†	
Water-soluble fraction							
Aspergillus oryzae (1926, med. N)	14.8	4.52	2.65	54.2	14.4	0.22	3.3
Trichoderma lignorum	38.3	6.78	3.50	51.6	24.3	0.15	5.6
Lycoperdon pyriforme	34.2	5.66	2.45	43.6	22.8	0.10	6.1
Clitocybe multiceps	57.2	8.42	3.95	46.9	30.2	0.35	6.8
Cottonseed meal	10.7	1.11	0.45	40.3	8.75	0.33	9.6
Alfalfa hay	50.0	4.07	1.15	28.3	29.5	0.37	12.3
Water-insoluble fraction							
Cottonseed meal	56.5	8.89	4.5	50.9	29.8	0.28	6.3
Lycoperdon pyriforme	60.2	4.38	0.92	21.0	29.4	1.79	11.5
Trichoderma lignorum	48.2	3.22	0.80	24.9	28.9	2.02	14.9
Aspergillus oryzae (1926, med. N)	92.5	5.48	1.45	26.4	61.7	2.46	17.0
Alfalfa hay	119.3	5.93	0.56	9.6	59.8	2.00	20.1
Clitocybe multiceps	51.3	1.58	−0.71	None	33.7	9.06	32.4
Alkali-insoluble fraction							
Lycoperdon pyriforme	35.8	3.4	1.27	37.6	22.9	1.03	10.5
Clitocybe multiceps	40.3	1.6	−0.29	None	26.9	4.37	25.2

— means less than the control.
* Total minus that of the control.
† Carbon evolved as carbon dioxide during the last 72-hour period.

formation were going on as rapidly as the process of ammonification and thus preventing any accumulation of ammonia. The figures for nitrate nitrogen at 10 days show this very clearly. In the case of ammonium sulfate 90 per cent of the nitrogen added had gone over to nitrate and the nitrogen from the organic sources to the extent of 25 to 60 per cent. These data show that the slow accumulation of nitrate nitrogen is not due primarily so much to a

retarded nitrate formation as to a slow liberation of ammonia not used by microorganisms.

At 26 days the amounts of nitrate nitrogen had increased approximately 10 p.p.m. over those present at 10 days. A few of the low nitrogen fungous tissues gave little nitrification but most of them showed a mineralization of from 30 to 60 per cent of their nitrogen content, which was as good as or better than that of the other organic nitrogen carriers used. Straw and timothy hay gave a depression of nitrate nitrogen. When the materials were separated into their water-solubility fractions the water-soluble fraction always gave a greater and the water-insoluble fraction a lesser percentage of nitrate accumulation than the total, and in each case the sum of the nitrate nitrogen obtained from the two fractions approximate that from the total material.

THE RELATION OF CARBONACEOUS ORGANIC MATTER TO NITRATE ACCUMULATION

Amount of Carbon

When ammonium sulfate is introduced into a soil it is usually rather readily changed to nitrate, but if there is an available energy material present, less nitrogen will appear as nitrate in a given time. In this same way the carbon combined with organic nitrogen is a very definite factor in the mineralization of the nitrogen. The carbon-nitrogen ratio is an expression of the relative amounts of carbon and nitrogen in a substance and has often been used as an index of the availability of the nitrogen in an organic material. Batham (11), studying the mineralization of the nitrogen in pure organic compounds, found that the nitrate liberated is inversely proportional to the ratio of carbon to nitrogen in the substance. Concerning the relation of this ratio to the mineralization of organic nitrogen, Whiting (123) says: "With a high carbon content and a low nitrogen content nitrates may be produced slowly or not at all or they may be produced rapidly provided the carbon is not in a resistant state and the nitrogen of average to high content." This would indicate that not only the ratio of carbon to nitrogen but also the availability of the energy material is a factor in the mineralization of organic nitrogen. Tables 11 and 12 are arranged with the ratios of carbon to nitrogen in ascending order for the various groups of materials. These data show an inverse relationship between the carbon-nitrogen ratio and the mineralization process. A ratio higher than 11 or 12 to 1 gives a mineralization for 26 days of less than 30 per cent of the organic nitrogen. A lower ratio gives a somewhat higher nitrate accumulation. This relation seems to hold true with the fungous and other organic materials as well as with their water-solubility fractions. The ratio of carbon to nitrogen is not, however, truly inversely proportional to the nitrate accumulation, but rather a ratio of 12 to 1 seems to be about the dividing line between fair and little nitrate accumulation for a period of 26 days. This is shown by the fact that in 26 days, 35.5 per cent of the nitrogen was liberated from the tissue of *Aspergillus oryzae* with a carbon-nitrogen ratio of

10.7, 36.2 per cent from the tissue of *Secotium acuminatum* with a ratio of 5.3, and 30.5 per cent from that of a *Coprinus* sp. with a ratio of 6.6. These figures indicate that there are other factors operating besides that of the ratio of carbon to nitrogen.

The rate and amount of carbon evolved as carbon dioxide have been taken as a measure of biological activities in the soil and attempts have been made to correlate this with the mineralization process. The figures in table 11 for the amounts of carbon evolved as carbon dioxide from the various substances and fungous tissues indicate an inverse relation between this carbon and the amounts of nitrogen changed to nitrate, or a parallel with the ratio of carbon to nitrogen. This is true only in a general way and there are many exceptions

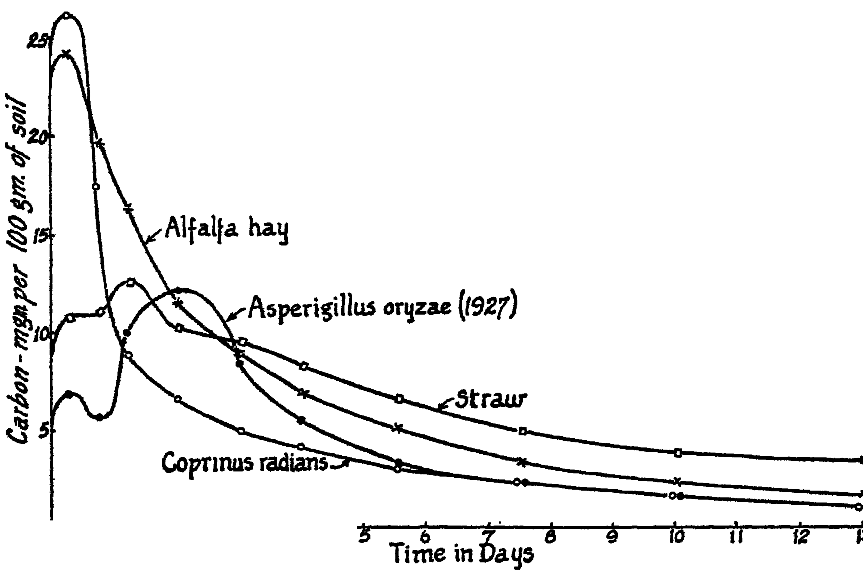


FIG. 3. RATE OF EVOLUTION OF CARBON DIOXIDE FROM VARYING AMOUNTS (10 MGM NITROGEN) OF DECOMPOSING ORGANIC MATTER IN MOIST SOIL

and some evidence to support the opposite view, especially in the case of a low nitrogen accumulation or of a depression. In the case of a depression, a lack of available nitrogen may cause retarded decomposition. In addition to the quantity of carbonaceous material present and the amount of carbon evolved as carbon dioxide for a given unit of nitrogen, there is evidence that the kind of carbonaceous material is also a factor in the mineralization process.

Kind of carbonaceous material

When organic matter decomposes in a soil a large amount of the carbon is evolved during the first few days. Figure 3 shows typical evolution curves for four of the materials used. All of these materials show the rapid develop-

ment of an early peak at about 12 hours with a more or less distinct second peak coming at from one to three days and then a gradual dropping off toward the control. With straw and the tissue of *Aspergillus oryzae* the second peak

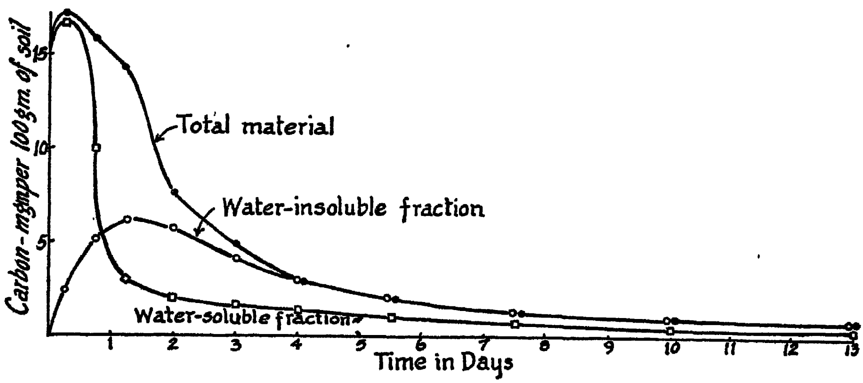


FIG. 4. EVOLUTION OF CARBON DIOXIDE FROM AN AMOUNT OF THE TISSUE OF LYCOPERDON PYRIFORME CARRYING 10 MGM. OF NITROGEN OR ITS WATER-SOLUBILITY FRACTIONS, WHEN DECOMPOSING IN MOIST SOIL

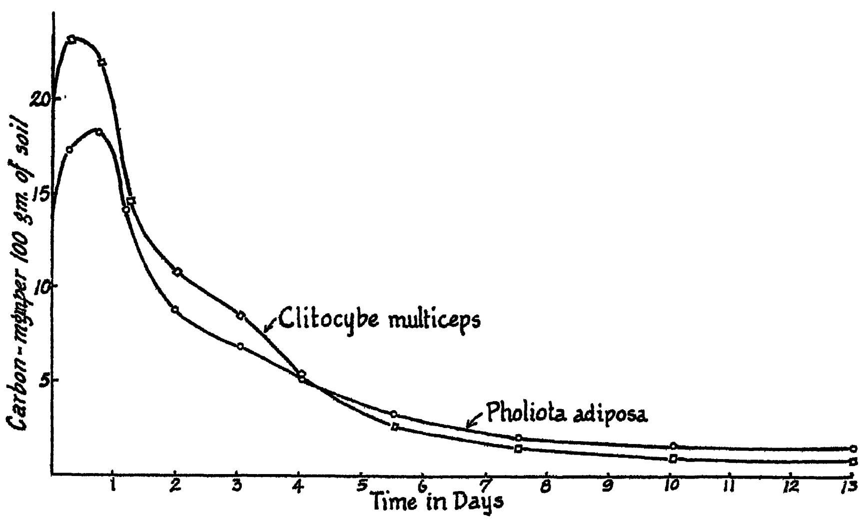


FIG. 5. EVOLUTION OF CARBON DIOXIDE FROM AMOUNTS OF TISSUE OF CLITOCYBE MULTICEPS AND PHOLIOTA ADIPOSA CARRYING 10 MGM. OF NITROGEN, WHEN DECOMPOSING IN MOIST SOIL

is well developed and the first one not so high, but with alfalfa and the tissue of *Coprinus radians* the first peak is high and well developed and the second one rather indistinct. The shape of these curves is indicative of the activities of soil microorganisms during a particular time interval and their height, the

intensity of this activity. The varying shapes and the double peaks indicate that the chemical composition or the chemical nature especially of the carbonaceous material is also variable. When the tissue of *Lycoperdon pyriforme* was separated into its fractions, each gave a distinctly different carbon evolution curve. Figure 4 shows that the water-soluble fraction gives an early peak with a rapid drop approaching the control, whereas the water-insoluble fraction gives a curve with a late peak and a slower decline toward the control. The sum of the carbon evolved from the two fractions gives a curve approximating that of the total.

The height of the evolution curves for carbon dioxide at the end of 26 days varies with the substances in question and is roughly in proportion to the ratio of carbon to nitrogen when a given amount of nitrogen is considered. Tables 11 and 12 show the amounts of carbon evolved as carbon dioxide during the last 72-hour period from an amount of material carrying 10 mgm. of nitrogen. It appears that the greater the amount of carbon evolved at this time the less the amount of nitrogen found as nitrate, and when this amount reaches approximately 1.7 to 2.0 mgm. of carbon for this period there is little or no nitrate accumulation. Without doubt the kind of energy material makes this difference. In table 11 in the cases of *Clitocybe multiceps* and *Pholiota adiposa*, the nitrogen contents of the tissues, the carbon-nitrogen ratios, and the total amounts of carbon evolved as carbon dioxide for 26 days are approximately the same, but from the former 27.5 per cent of its nitrogen is liberated as nitrate whereas from the latter there is a depression below the control. Figure 5 shows that the evolution curves are different, the former being high at the beginning and low at the end while the latter is just the reverse giving an evolution during the last 72 hours of 1.75 mgm. of carbon as against 0.90 mgm. for the former.

Thus it appears that the structure of the carbonaceous energy material governs somewhat the type of decomposition curves and at the same time the amount of nitrogen liberated as nitrate. If the energy materials are of simple nature their decomposition is rapid and their effect soon lost, but if they are of cellulosic form the decomposition is much slower and the action of the soil microorganisms is continued much longer with a corresponding inhibition in the accumulation of nitrate nitrogen. Allison (6) and Doryland (26) both felt that the energy-nitrogen relations are more important than the carbon-nitrogen relations as such, in that the rate of decomposition of the energy material depends much on its form and availability to microorganisms. Wilson and Wilson (126) have shown that with the same quantities of corn and sorghum roots in the soil, the depression of nitrate is different. In the early stages of decomposition the sorghum roots evolve more carbon dioxide than the corn roots and at the same time depress the nitrates to a greater extent. This they attribute to the greater amount of soluble energy material in the sorghum roots causing a greater biological activity. If the process is carried on longer than 30 days the reverse is true, more carbon dioxide is evolved from

the corn roots with a greater depression of nitrates than with the sorghum roots.

Type of organism

The form or kind of energy materials determines to a great extent the kind of organisms predominating in the decomposition. If these materials are

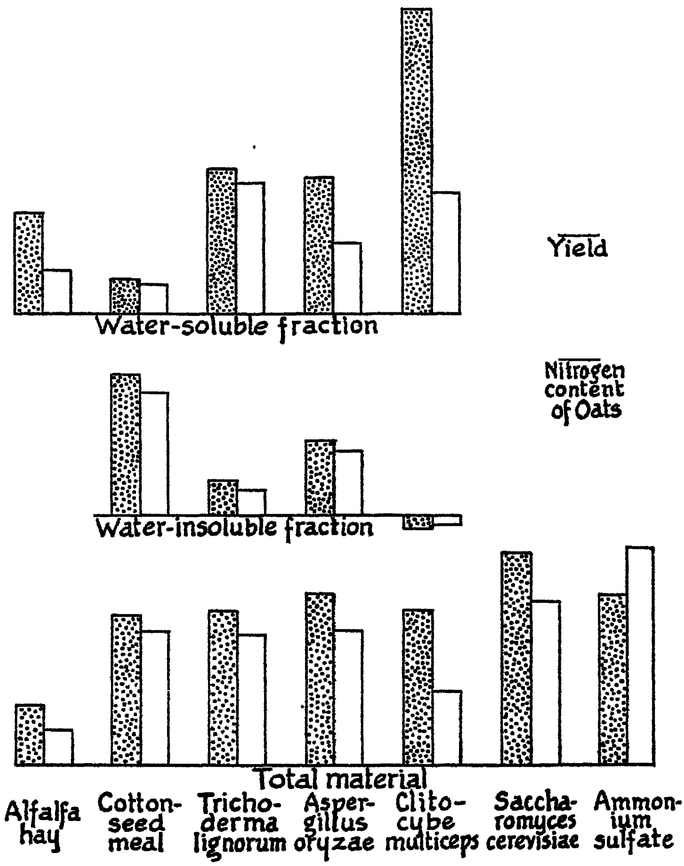


FIG. 6. RELATIVE YIELDS AND AMOUNTS OF NITROGEN ASSIMILATED BY OATS FROM FUNGOUS TISSUE AND OTHER NITROGEN CARRIERS (400 MGM. OF NITROGEN OR THE SOLUBILITY FRACTIONS WERE USED IN EACH CASE)

simple the decomposition will be largely bacterial and quickly completed, whereas if they are cellulosic the action will be largely fungous and of longer duration. The curve for the water-soluble fraction in figure 4 is typical for that of bacteria and that for the water-insoluble fraction for the fungi. These facts correlate with the observations of the fungous growth and bacterial counts as previously noted. Bacterial decomposition in the soil is very

complete and most of the carbon may be accounted for as carbon dioxide. The fungi, on the other hand, are more efficient in their use of carbon, for it has been shown (48) that as much as 40 per cent of the carbon used by fungi is found in their mycelial tissue. Neller (77) found in the decomposition of alfalfa meal, that fungi gave a greater evolution of carbon dioxide than bacteria, but that the latter showed a higher ammonia accumulation. This indicates that the bacteria attack the simpler carbonaceous compounds, build up very little protoplasm, and liberate much of the nitrogen as ammonia, whereas the fungi attack the cellulosic portion of the alfalfa meal with the production of much fungous tissue in which they use practically all of the nitrogen liberated in the decomposition process especially if the ratio of carbon to nitrogen is greater than 12 to 1.

On the whole it appears that the mineralization of organic nitrogen depends not only on the amount of carbonaceous or energy material with it, but also on the form and availability of this material and the resultant microflora. A given amount of energy material when used by soil organisms requires a given amount of nitrogen for its decomposition and this nitrogen is built up into the organic form in the microbial substance. Any nitrogen in excess of this amount is liberated in the mineral form. If the energy material is of a simple soluble nature, the decomposition is largely bacterial with a rapid evolution of the carbon as carbon dioxide. In this case a small amount of microbial protoplasm is formed with the result that a large amount of organic nitrogen is quickly changed to the inorganic form. If on the other hand the same amount of energy material is in a cellulosic form the decomposition is largely by fungi with a slower use of energy material extending over a longer period of time. These conditions lead to the formation of larger quantities of microbial substance than with the simpler energy materials and the use of greater quantities of nitrogen resulting in a lower nitrate accumulation. At the same time the effect of the energy extends over a longer period, maintaining the organisms in the living state and preventing the liberation of their nitrogen through autolysis or decomposition.

GROWTH OF OATS FROM FUNGOUS NITROGEN

In the previous work it has been found that the nitrogen in fungous tissue is readily changed to the mineral form in the soil. A series of plant cultures was arranged to test the ability of higher plants to use this nitrogen. Oats were used and the soil was the same as in the previous experiment. Here 10 kgm. of soil were used in a 2-gallon pot and 1 gm. of mono-potassium phosphate was added to each. The fungous tissues and other organic nitrogen carriers were prepared in the same way as described above. The total tissue was added in such an amount that it represented 400 mgm. of nitrogen per pot, and the water-soluble and water-insoluble portions carried the fractions of that amount that were soluble or insoluble in water. The oats were thinned to 12 plants per pot and watered with distilled water. The length of the growing period

was approximately 8 weeks. The tops only were harvested just before the heading stage, dried, ground, and the total nitrogen determined. Table 13 shows the results of the oat culture study including the yields and nitrogen recovery in the crop. The figures given are the averages for duplicate cultures. Plate 1 shows the oats at the time of harvest.

TABLE 13

The use by oats of nitrogen from decomposing fungous tissue and other nitrogen carriers

MATERIAL	MATERIAL ADDED PER POT			DRY WEIGHT, OATS PER POT	NITROGEN CONTENT OF OATS		NITROGEN RECOVERED IN OATS
	Total	Carbon	Nitrogen				
	gm.	*mgm.*	*mgm.*	*gm.*	*per cent*	*mgm.*	*per cent*
Controls	......	......	......	7.35	1.17	86.0	.....
Ammonium sulfate	1.90	....	400	12.5	2.85	356.2	67.5
Alfalfa hay, total	15.95	6,767	400	9.2	1.41	129.7	10.9
Alfalfa hay, water-soluble	....	2,000	162.7	10.45	1.33	139.0	32.5
Alfalfa hay, water-insoluble	....	4,767	237.3	7.15	1.33	95.1	3.9
Cottonseed meal, total	6.024	2,687	400	12.05	2.09	252.4	41.6
Cottonseed meal, water-soluble	....	427	44.7	8.45	1.44	121.7	80.0
Cottonseed meal, water-insoluble	....	2,260	355.3	12.1	1.96	237.2	42.3
Trichoderma lignorum, total	7.80	3,460	400	12.1	2.04	246.8	40.2
Trichoderma lignorum, water-soluble	....	1,533	271.3	11.85	2.11	249.9	60.4
Trichoderma lignorum, water-insoluble	....	1,927	128.7	8.4	1.40	117.6	24.8
Aspergillus oryzae (1927), total	8.7333	3,660	400	12.65	1.84	232.7	36.7
Aspergillus oryzae (1927), water-soluble	....	390.4	120.5	11.55	1.49	172.1	71.4
Aspergillus oryzae (1927), water-insoluble	....	3,269.6	279.5	9.7	1.71	165.9	28.6
Clitocybe multiceps, total	10.70	4,340	400	12.0	1.48	177.6	22.9
Clitocybe multiceps, water-soluble	....	2,287	336.7	16.8	1.41	236.9	44.8
Clitocybe multiceps, water-insoluble	....	2,053	63.3	6.95	1.22	84.8	−1.9
Saccharomyces cerevisiae, total	4.20	1,840	400	13.85	2.08	288.1	50.5

The water-soluble nitrogen has the highest availability of any fraction of the nitrogen. The range is from 40 to 70 per cent and is in accord with earlier experiments reported in this work. The high availability of the water soluble nitrogen is largely due to three factors: first, low amount of energy material or low carbon-nitrogen ratio; second, carbonaceous material which is readily decomposed and largely by bacterial action; and third, simple form of nitrogen.

Figures already given show that over half of the nitrogen in the water-soluble fraction is in the free amino form. This, together with its high dializability, indicates that the water-soluble nitrogen is very simple and easily decomposed. In such material the energy carbon is quickly evolved as carbon dioxide and little bacterial protoplasm is built up, thus liberating much of the organic nitrogen in the mineral form.

TABLE 14

A comparison of the availability of nitrogen in fungous tissue and other nitrogen carriers as measured by nitrification and oat culture experiments

MATERIAL	AVAILABILITY OF NITROGEN	
	In nitrification experiment	In oat culture experiment
	per cent	*per cent*
Ammonium sulfate	93.5	67.5
Alfalfa hay, total	16.5	10.9
Alfalfa hay, water-soluble	28.3	32.5
Alfalfa hay, water-insoluble	9.6	3.9
Cottonseed meal, total	49.5	41.6
Cottonseed meal, water-soluble	40.3	80.0
Cottonseed meal, water-insoluble	50.9	42.4
Trichoderma lignorum, total	39.5	40.2
Trichoderma lignorum, water-soluble	51.6	60.4
Trichoderma lignorum, water-insoluble	24.9	24.8
Aspergillus oryzae (1927), total	42.0	36.7
Aspergillus oryzae (1927), water-soluble	....	71.4
Aspergillus orysae (1927), water-insoluble	....	28.6
Clitocybe multiceps, total	27.5	22.9
Clitocybe multiceps, water-soluble	46.9	44.8
Clitocybe multiceps, water-insoluble	−44.8	−1.9
Saccharomyces cerevisiae, total	59.5	50.5

— means less than the control.

The water-insoluble portion of all materials except cottonseed meal has a low availability, sometimes giving a depression of nitrate nitrogen. This is due not only to the high ratio of carbon to nitrogen but also to the kind of energy material present in the tissue. In this fraction the energy material is largely of the cellulosic type, which supports the growth of fungi to a much greater extent than bacteria. The fungi have a greater efficiency in the use of carbon and thus build up more carbon and at the same time more nitrogen into organic form.

The percentage availability of the nitrogen in the total tissue usually falls between those of the water-soluble and water-insoluble fractions. The amount of nitrate produced in the decomposition of fungous tissue is much the same as that produced from other organic nitrogen carriers of a similar nitrogen content. The amount of nitrogen in most of the fungous tissue studied ranged from 4 to 7 per cent and the availability of this nitrogen compared favorably with that of cottonseed meal which had a nitrogen content of 6.64 per cent. The species produced on artificial liquid culture media had a slightly higher amount of available nitrogen than did the natural forms, perhaps because of their slightly greater solubility in water.

There was a very close parallel between the percentages of the nitrogen changed to nitrate in the nitrification work and the amount of nitrogen found in the tops of the oat plants. Table 14 gives these data, and figure 6 shows graphically the relative yields of oats and the amounts of nitrogen assimilated by the crop. With the exception of the ammonium sulfate, a part of which no doubt was used in base exchange, the water-soluble portion of cottonseed meal, and the water-insoluble portion of *Clitocybe multiceps*, the latter two carrying low amounts of nitrogen, the parallel is almost perfect. As a rule the nitrogen obtained by the oats was slightly lower than the percentage of nitrification obtained in previous studies. Although the growth period of the oats was longer than the nitrification period, the tops of the oats only were harvested which would help to account for the slightly lower assimilation of nitrogen by the oats.

SUMMARY

A study was made of the nature and availability of the nitrogen in fungous tissue and in various organic materials. Fungous tissue was collected from natural habitats and also grown on artificial liquid culture media. The fungous material was separated into various chemical fractions and a study made of the carbon and nitrogen in these fractions as well as in the total material. The decomposition and nitrification of the various fungous tissues were studied in relation to carbon dioxide evolution and nitrate accumulation and also the effect of these tissues on the growth of higher plants.

The results of this work may be summarized as follows:

1. The carbon content of all fungous tissue studied is rather constant, fluctuating for the most part only between 40 and 44 per cent. The nitrogen content of the fungi found growing in the fields and woods varies from 1.5 to over 7 per cent, and the majority of these forms contain more than 4 per cent of nitrogen on a dry basis. In mycelium produced on synthetic liquid media, the nitrogen varied from less than 2 per cent to more than 6 per cent for the same species. The energy nitrogen ratio of the substrate is the determining factor, both in the quantity of the mycelium produced and the amount of nitrogen it contains. As the available nitrogen in the substrate decreases, the nitrogen content of the mycelium decreases to about 2 or 3 per cent. At this point a further decrease in the amount of nitrogen in the substrate causes a decrease in the weight of the mycelial tissue, so that the nitrogen content of the tissue seldom falls below 2 per cent. The decrease in the amount of the fungous tissue produced begins when its carbon-nitrogen ratio reaches 10 or 12 to 1.

2. Fungous nitrogen is for the most part very simple. From 40 to 70 per cent of the nitrogen in the dry fungous tissue used in this work was soluble in water, and of this portion, 80 to 92 per cent was dializable through a collodion sack. From 80 to 85 per cent of the total nitrogen was soluble in 0.05 *N* sodium hydroxide solution in 60 per cent alcohol. From 40 to 65 per cent of the nitrogen in the water-soluble and alcohol-soluble fractions was free amino nitrogen. The alcohol-soluble fraction was more complex, being only about 10 to 15 per cent free amino nitrogen. No urea was found in the tissue tested.

3. Most fungous tissues decompose readily in moist soils. From 40 to 60 per cent of their carbon is liberated as carbon dioxide in 26 days. On decomposition the nitrogen which they contain is liberated as nitrate to the extent of from 30 to 42 per cent of the original amount during a period of 26 days. The balance is either not liberated or is again combined into a new fungous or bacterial substance. When there is no other energy material present, living fungous tissue liberates its own nitrogen by autolysis to even a greater extent than the dead tissue. The rate of mineralization of fungous nitrogen depends upon the amount and kind of energy materials present. When the energy material is simple the decomposition is largely bacterial with the liberation of large amounts of nitrate nitrogen, but when it is of a cellulosic nature, the action is to a great extent fungous with the liberation of little or no mineral nitrogen. The nitrogen in fungous tissue in the soil is as readily nitrified as, or even more rapidly nitrified than that of other organic materials of similar nitrogen content.

REFERENCES

(1) Abbott, E. V. 1923 The occurrence and action of fungi in soils. *Soil Sci.* 16: 207–216.

(2) Abbott, E. V. 1926 Taxonomic studies of soil fungi. *Iowa State Col. Jour. Sci.* 1: 15–36.

(3) Abbott, E. V. 1926 A study of microbiological activities in some Louisiana soils. La. Agr. Exp. Sta. Bul. 194.

(4) Adametz, L. 1886 Untersuchungen über die niederen Pilze der Ackerkrume. *Inaug. Diss. Leipzig: Centbl. Bakt.* (1) 1: 8–10 (1887).

(5) Albrecht, W. A. 1926 Nitrate accumulation in soil as influenced by tillage and straw mulch. *Jour. Amer. Soc. Agron.* 18: 841–853.

(6) Allison, F. E. 1927 Nitrate assimilation by soil microörganisms in relation to available energy supply. *Soil Sci.* 24: 79–93.

(7) Association of Official Agricultural Chemists. 1925 Official and tentative methods of analysis compiled by the committee on editing methods of analysis, ed. 2. Washington, D. C.

(8) Barthel, C., and Bengtsson, N. 1924 Action of stable manure in the decomposition of cellulose in tilled soil. *Soil Sci.* 18: 185–200.

(9) Barthel, C., and Bengtsson, N. 1926 Bidrag till Frågen om Stallgödselkävets nitrifikation i åkerjorden. Meddel. Centralanst. Försöksv. Jordbruksområdet [Sweden] 311.

(10) Barthel, C., and Bengtsson, N. 1927 Availability of the nitrogen in fungi and bacterial cells for nitrification and cellulose decomposition in the soil. *Proc. First Internatl. Congr. Soil Sci.* 2: 204–208.

(11) Batham, H. N. 1927 Nitrification in soils: II. *Soil Sci.* 24: 187–203.

(12) Bierema, S. 1909 Die Assimilation von Ammon-, Nitrat- und Amidstickstoff durch Mikroorganismen. *Centr. Bakt.* (2) 23: 672–726.

(13) Blair, A. W., and Prince, A. L. 1928 The influence of heavy applications of dry organic matter on crop yields and on the nitrate content of the soil. *Soil Sci.* 25: 281–287.

(14) Brach, H. 1912 Untersuchungen über den chemischen Aufbau des Chitins. *Biochem. Ztschr.* 38: 468–491.

(15) BRACONNOT, H. 1811 De la Fongine, ou analyse des champignons. *Jour. Phys., Chim.* etc. 73: 130. From Lafar, Technical Mycology, v. 2, London, 1911.
(16) CARPENTIER, C. A. G. 1921 Studier över Stallgödselns inverkan på cellulosans sönderdelning i åkerjord. (Studies on the effect of manure on cellulose decomposition.) Meddel. Centralanst. Försöksv. Jordbruksområdet. [Sweden] 218. *Abs. Bact.* 6: 182 (1922).
(17) CHAMBERS, C. O. 1916. The fixation of free nitrogen by certain fungi. *Plant World* 19: 175–194.
(18) CLARK, E. D., AND SCALES, F. M. 1916 Enzymes of a cellulose-destroying fungus from the soil, *Penicillium pinophilum. Jour. Biol. Chem.* 24: xxxi.
(19) COLEMAN, D. A. 1916 Environmental factors influencing the activity of soil fungi. *Soil Sci.* 2: 1–65.
(20) COLLISON, R. E., AND CONN, H. J. 1925 The effect of straw on plant growth. N. Y. Agr. Exp. Sta. Tech. Bul. 114.
(21) CONN, H. J. 1916 Relative importance of fungi and bacteria in soil. *Science* 44: 857–858.
(22) CONN, H. J. 1922 A microscopic method for demonstrating fungi and actinomycetes in soil. *Soil Sci.* 14: 149–151.
(23) DALE, E. 1912 On the fungi of the soil. *Ann. Mycol.* 10: 452–477.
(24) DALE, E. 1914 On the fungi of the soil. *Ann. Mycol.* 12: 33–62.
(25) DORE, W. H., AND MILLER, R. C. 1923 The digestion of wood by *Teredo navalis. Cal. Univ. Pub. Zool.* 22 (6): 383–400.
(26) DORYLAND, C. J. T. 1916 The influence of energy material upon the relation of soil microorganisms to soluble plant food. N. D. Agr. Exp. Sta. Bul. 116.
(27) DOX, A. W. 1909 The intracellular enzymes of lower fungi, especially those of *Penicillium camemberti. Jour. Biol. Chem.* 6: 461–467.
(28) DOX, A. W. 1912 Enzyme studies of lower fungi. *Plant World* 15: 40–43.
(29) DOX, A. W. 1913 Autolysis of mold cultures: II. Influence of exhaustion of the medium upon the rate of autolysis of *Aspergillus niger. Jour. Biol. Chem.* 16: 479–484.
(30) DOX, A. W. 1915 The soluble polysaccharides of lower fungi: III. The influence of autolysis on the mycodextran content of *Aspergillus niger. Jour. Biol. Chem.* 20: 83–85.
(31) DOX, A. W., AND GOLDEN, R. 1911 Phytase in lower fungi. *Jour. Biol. Chem.* 10: 183–186.
(32) DOX, A. W., AND MAYNARD, L. 1912 Autolysis of mold cultures. *Jour. Biol. Chem.* 12: 227–231.
(33) DOX, A. W., AND NEIDIG, R. E. 1911 Pentosans in lower fungi. *Jour. Biol. Chem.* 9: 267–269.
(34) DOX, A. W., AND NEIDIG, R. E. 1914 The soluble polysaccharides of lower fungi: I. Mycodextran, a new polysaccharide in *Penicillium expansum. Jour. Biol. Chem.* 18: 167–175.
(35) DOX, A. W., AND NEIDIG, R. E. 1914 The soluble polysaccharides of lower fungi. II. Mycogalactan, a new polysaccharide in *Aspergillus niger. Jour. Biol. Chem.* 19: 235–237.
(36) DOX, A. W., AND ROARK, JR., G. W. 1920 The utilization of α-methylglucoside by *Aspergillus niger. Jour. Biol. Chem.* 41: 475–481.
(37) DUGGAR, B. M., AND DAVIS, A. R. 1916 Studies in the physiology of the fungi: I. Nitrogen fixation. *Ann. Missouri Bot. Gard.* 3: 413–437.
(38) FALCK, R. 1923 Mykologische Untersuchungen und Berichte, v. 2, Geb. Cassel., p. 15.
(39) FLEMING, W. E. 1925 The relation of fungi to the numbers of bacteria in the soil. *Soil Sci.* 19: 301–307.

(40) Frémy, M. E. 1859 Recherches sur la composition chimique des tissus des végétaux. *Jour. Pharm. et Chim.* 336: 5–14.

(41) Gilman, J. C., and Abbott, E. V. 1927 A summary of the soil fungi. *Iowa State Col. Jour. Sci.* 1: 225–345.

(42) Goddard, H. N. 1913 Can fungi living in agricultural soil assimilate free nitrogen? *Bot. Gaz.* 56: 249–305.

(43) Harper, H. J. 1924 The determination of ammonia in soils. *Soil Sci.* 18: 409–418.

(44) Harper, H. J. 1924 The accurate determination of nitrates in soils. *Indus. Engin. Chem.* 16: 180–183.

(45) Hawkins, L. A. 1915 The utilization of certain pentoses and compounds of pentoses by *Glomerella cingulata*. *Amer. Jour. Bot.* 2: 375–388.

(46) Hawley, L. F., Fleck, L. C., and Richards, C. A. 1928 Effect of decay on the chemical composition of wood. *Indus. Engin. Chem.* 20: 504–507.

(47) Heukelekian, H. 1924 Decomposition of cellulose by the various groups of microorganisms of the soil. *Abs. Bact.* 8: 9.

(48) Heukelekian, H., and Waksman, S. A. 1925 Carbon and nitrogen transformations in the decomposition of cellulose by filamentous fungi. *Jour. Biol. Chem.* 66: 323–342.

(49) Ishida, M., and Tollens, B. 1911 Uber die Bestimmung von Pentosan und Methyl-Pentosan in Getreide und in Holzpilzen. *Jour. Landw.* 59: 59–67.

(50) Iterson, Jr., C. van. 1904 Die Zersetzung von Cellulose durch aërobic Mikroorganismen. *Centbl. Bakt.* (2) 11: 689–698.

(51) Iwanoff, N. N. 1923 Über den harnstoffgehalt der Pilze. *Biochem. Ztschr.* 136: 1–8.

(52) Iwanoff, N. N. 1923 Über die bildung des harnstoffs in Pilzen. *Biochem. Ztschr.* 136: 9–19.

(53) Iwanoff, N. N. 1923 Über das Viscosin der Pilze. *Biochem. Ztschr.* 137: 320–330.

(54) Iwanoff, N. N. 1923 Über die Nature des Eiweiszstoffes der Pilze. *Biochem. Ztschr.* 137: 331–340.

(55) Iwanoff, N. N. 1924 Über die Ursache des verschiedenen Harnstoffgehalts in Pilzen. *Biochem. Ztschr.* 154: 391–398.

(56) Iwanoff, N. N. 1928 Über Harnstoff in Pilzen. *Biochem. Ztschr.* 192: 36–40.

(57) Iwanoff, N. N., and Toschewikowa, A. 1927 Über zwei Arten von Harnstoffbildung bei Champignons. *Biochem. Ztschr.* 181: 1–7.

(58) Jensen, C. N. 1912 Fungous flora of the soil. N. Y. (Cornell) Agr. Exp. Sta. Bul. 315.

(59) Kotake, Y., and Sera, Y. 1913 Über eine neue Glukosaminverbindung, zugleich ein Beitrag zur Konstitutionsfrage des Chitins. *Ztschr. Physiol. Chem.* 88: 56–72.

(60) Klein, G., Eigner, A., and Müller, H. 1926 Nitratassimilation bei Schimmelpilzen. *Ztschr. Physiol. Chem.* 159: 201–234.

(61) Klotz, L. J. 1923 Studies in the physiology of the fungi. XVI. Some aspects of nitrogen metabolism in fungi. *Ann. Missouri Bot. Gard.* 10: 299–368.

(62) Kopeloff, N. 1916 The inoculation and incubation of soil fungi. *Soil Sci.* 1: 381–403.

(63) Kossowicz, A. 1914 Zur Frage der Assimilation des elementaren Stickstoffs durch Hefen und Schimmelpilze. *Biochem. Ztschr.* 64: 82–85.

(64) Kress, O., et al. 1925 Control of decay in pulp and pulp wood. U. S. Dept. Agr. Bul. 1298.

(65) Lafar, F. 1904–1907 Handbuch der Technischen Mykologie, v. 5. Jena.

(66) LIPMAN, C. B. 1911 Nitrogen fixation by yeasts and other fungi. *Jour. Biol. Chem.* 10: 169–182.
(67) LYON, T. L. 1926 Nitrates in the soil as influenced by the growth of plants. *Jour. Amer. Soc. Agron.* 18: 834–840.
(68) LYON, T. L., BIZZELL, J. A., AND WILSON, B. D. 1923 Depressive influence of certain higher plants on the accumulation of nitrates in soil. *Jour. Amer. Soc. Agron.* 15: 457–467.
(69) MCBETH, I. G., AND SCALES, F. M. 1913 The destruction of cellulose by bacteria and filamentous fungi. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266.
(70) MCLEAN, H. C., AND WILSON, G. W. 1914 Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270.
(71) MCLENNAN, E. 1928 The growth of fungi in soil. *Ann. Appl. Biol.* 15: 95–109.
(72) MARSHALL, JR., E. K. 1913 A rapid clinical method for the estimation of urea in urine. *Jour. Biol. Chem.* 14: 283–290.
(73) MAZÉ, P. 1902 Recherches sur les modes d'utilisation du carbone ternaire par les végétaux et les microbes. *Ann. Inst. Past.* 16: 346–378; 433–451.
(74) MURRAY, T. J. 1921 The effect of straw on the biological soil processes. *Soil Sci.* 12: 233–259.
(75) MÜTTERLEIN, C. 1913 Studien über die Zersetzung der Cellulose im Dünger und im Boden. Univ. Leipzig Diss.
(76) NEIDIG, R. E. 1913 Polyatomic alcohols as sources of carbon for lower fungi. *Jour. Biol. Chem.* 16: 143–145.
(77) NELLER, J. R. 1918 Studies on the correlation between the production of carbon dioxide and the accumulation of ammonia by soil organisms. *Soil Sci.* 5: 225–241.
(78) OUDEMANS, C. A. J. C., AND KONING, C. J. 1902 Prodrome d'une flore mycologique, obtenue par la culture sur gelatin preparée de la terre humeuse du spanderswoud près de Bussum. *Arch. Néerland. Sci. Exact. et Nat.* (2) 7: 266–298.
(79) PAINE, F. S. 1927 Studies of the fungous flora of virgin soils. *Mycologia.* 19: 248–267.
(80) PETERSON, W. H., FRED, E. B., AND SCHMIDT, E. G. 1922 The fermentation of pentoses by molds. *Jour. Biol. Chem.* 54: 19–34.
(81) PRATT, O. A. 1918 Soil fungi in relation to diseases of the Irish potato in southern Idaho. *Jour. Agr. Res.* 13: 73–100.
(82) PRINGSHEIM, H. 1910 Studien über die Spaltung racemischer Aminosaüren durch Pilze. *Ztschr. Physiol. Chem.* 65: 96–109.
(83) PRINGSHEIM, H., AND ZEMPLEN, G. 1909 Studien über die Polysaccharide spaltenden Fermente in Pilzpreszsäften. *Ztschr. Physiol. Chem.* 62: 367–385.
(84) PROSKURIAKOW, N. J. 1926 Über die Beteiligung des Chitins am Aufbau der Pilzzellwand. *Biochem. Ztschr.* 167: 68–76.
(85) RAHN, O. 1919 Die schädliche Wirkung der Strohdüngung und deren Verhütung. *Ztschr. Tech. Biol.* 7: 172–186.
(86) RATHBUN, A. E. 1918 The fungous flora of pine seed beds. *Phytopathology* 8: 469–483.
(87) REGE, R. D. 1927 Biochemical decomposition of cellulosic materials, with special reference to the action of fungi. *Ann. Appl. Biol.* 14: 1–44.
(88) REUTER, C. 1912 Beiträge sur Kenntnis der Stickstoffhaltigen Bestandteile der Pilze. *Ztschr. Physiol. Chem.* 78: 167–245.
(89) RUSSELL, E. J. 1923 The Microorganisms of the Soil, chap. 7 and 8. London.
(90) SCALES, F. M. 1914 The enzymes of *Aspergillus terricola. Jour. Biol. Chem.* 19: 459–472.
(91) SCALES, F. M. 1915 Some filamentous fungi tested for cellulose destroying power. *Bot. Ga.* 60: 149–153.

(92) SCHENKER, R. 1921 Zur Kenntnis der Lipase von *Aspergillus niger* (van Tiegh). *Biochem. Ztschr.* 120: 164–196.

(93) SCHMIDT, E. G., PETERSON, W. H., AND FRED, E. B. 1923 The destruction of pentosans by molds and other microörganisms. *Soil Sci.* 15: 479–488.

(94) SCHRENK, H. VON. 1900 Two diseases of red cedar caused by *Polyporus juniperinus* n. sp. and *Polyporus carneus* Nees. U. S. Dept. Agr. Veg. Physiol. and Path. Bul. 21.

(95) SCOTT, H. 1921 The influence of wheat straw on the accumulation of nitrates in the soil. *Jour. Amer Soc. Agron.* 13: 233–258.

(96) SIEBER, N. 1881 Beiträge zur Kenntniss der chemischen Zusammensetzung der Schimmelpilze. *Jour. Prakt. Chem.* (N. F.) 23: 412–421.

(97) SIEVERS, F. J., AND HOLTZ, H. F. 1926 The significance of nitrogen in soil organic matter relationships. Wash. Agr. Exp. Sta. Bul. 206.

(98) SKINNER, C. E. 1925 What organisms are responsible for the decomposition of cellulose in the soil? *Abs. Bact.* 9: 32.

(99) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.* 17: 293–314.

(100) STARKEY, R. L. 1924 Evolution of carbon dioxide as an index of decompsotion of organic matter in the soil. *Abs. Bact.* 8: 9.

(101) STEINBERG, R. A. 1919 A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. *Amer. Jour. Bot.* 6: 330–372.

(102) TANRET, M. C. 1897 Recherches sur les champignons. *Bul. Soc. Chim. Paris* (3) 17: 921–927.

(103) THOM, C., AND LATHROP, E. C. 1925 Psilocybe as a fermenting agent in organic. debris. *Jour. Agr. Res.* 30: 625–628.

(104) VAN SLYKE, D. D. 1912 The quantitative determination of aliphatic amino groups. II. *Jour. Biol. Chem.* 12: 275–284.

(105) VAN SLYKE, D. D. 1913 The gasometric determination of aliphatic amino nitrogen in minute quantities. *Jour. Biol. Chem.* 16: 121–124.

(106) VILJOEN, J. A., AND FRED, E. B. 1924 The effect of different kinds of wood and wood pulp cellulose on plant growth. *Soil Sci.* 17: 199–211.

(107) VORBRODT, W. 1926 O przeróbce azotu w grzybni kropidlaka (*Aspergillus niger*). (Elaboration of nitrogen in the mycelium of *A. niger*.) *Bul. Internatl. Acad. Polonaise Sci. et Lett.* (*B*) *Sci. Nat.* (5/6B): 517–533. Abs. in *Biol. Abs.* 1 (11440): 1025 (1928).

(108) WAKSMAN, S. A. 1916 Soil fungi and their activities. *Soil Sci.* 2: 103–156.

(109) WAKSMAN, S. A. 1917 Is there any fungus flora of the soil? *Soil Sci.* 3: 565–589.

(110) WAKSMAN, S. A. 1918 Studies on proteolytic activities of soil microörganisms with special reference to fungi. *Jour. Bact.* 3: 475–492.

(111) WAKSMAN, S. A. 1918 Studies on the proteolytic enzymes of soil fungi and actinomycetes. *Jour. Bact.* 3: 509–530.

(112) WAKSMAN, S. A. 1922 The growth of fungi in the soil. *Soil Sci.* 14: 153–157.

(113) WAKSMAN, S. A. 1926 The origin and nature of the soil organic matter or soil "humus:" III. The nature of the substances contributing to the formation of humus. *Soil Sci.* 22: 323–333.

(114) WAKSMAN, S. A. 1926 On the origin and nature of the soil organic matter or soil "humus:" V. The rôle of microörganisms in the formation of "humus" in the soil. *Soil Sci.* 22: 421–436.

(115) WAKSMAN, S. A. 1927 Principles of Soil Microbiology. Baltimore.

(116) WAKSMAN, S. A., AND COOK, R. C. 1916 Incubation studies with soil fungi. *Soil Sci.* 1: 275–284.

(117) WAKSMAN, S. A., AND HEUKELEKIAN, O. 1924 Microbiological analysis of soil as an index of soil fertility: VIII. Decomposition of cellulose. *Soil Sci.* 17: 275–291.

(118) WAKSMAN, S. A., AND HEUKELEKIAN, H. 1926 Cellulose decomposition by various groups of soil microorganisms. *Actes de la IVeme Conf. Internatl. Pedologie* 3: 216–226.

(119) WAKSMAN, S. A., AND TENNEY, F. G. 1926 On the origin and nature of the soil organic matter or soil "humus:" IV. The decomposition of the various ingredients of straw and of alfalfa meal by mixed and pure cultures of microörganisms. *Soil Sci.* 22: 395–406.

(120) WAKSMAN, S. A., AND SKINNER, C. E. 1926 Microörganisms concerned in the decomposition of celluloses in the soil. *Jour. Bact.* 12: 57–84.

(121) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, actinomycetes, and bacteria in the soil. *Soil Sci.* 17: 373–378.

(122) WHITE, J. W., AND HOLBEN, F. J. 1925 Perfection of chromic acid method for determining organic carbon. *Indus. Engin. Chem.* 17: 83–85.

(123) WHITING, A. L. 1926 Some important factors controlling the rate of nitrification of organic materials. *Jour. Amer. Soc. Agr.* 18: 854–876.

(124) WHITING, A. L., AND RICHMOND, T. E. 1927 The relative rates of nitrification of different parts of sweet clover plants. *Soil Sci.* 24: 31–37.

(125) WICHERS, J. L., AND TOLLENS, B. 1910 III. Über die Pentosane einiger Holzpilze. *Jour. Landw.* 58: 238–242.

(126) WILSON, B. D., AND WILSON, J. K. 1928 Relation of sorghum roots to certain biological processes. *Jour. Amer. Soc. Agron.* 20: 747–754.

(127) WINTERSTEIN, E. 1893 Zur Kenntniss der Pilzcellulose. *Ber. Deut. Bot. Gesell.* 11: 441–445.

(128) WINTERSTEIN, E. 1894 Zur Kenntniss der in den Membranen der Pilze enthaltenen Bestandtheile. *Ztschr. Physiol. Chem.* 19: 521–562.

(129) WINTERSTEIN, E. 1894 Ueber ein stickstoffhaltiges Spaltungsproduct der Pilzecellulose. *Ber. Deut. Chem. Gesell.* 27: 3113–3115.

(130) WINTERSTEIN, E. 1895 Zur Kenntniss der in den Membranen der Pilze enthaltenen Bestandtheile. *Ztschr. Physiol. Chem.* 21: 134–151.

(131) WINTERSTEIN, E. 1895 Ueber Pilzcellulose. *Ber. Deut. Bot. Gesell.* 13: 65–70.

(132) WINTERSTEIN, E., AND REUTER, C. 1912 Über die Stickstoffhaltigen Bestandteile der Pilze. *Centbl. Bakt.* (2) 34: 566–573.

(133) WINTERSTEIN, E., REUTER, C., AND KOROLEW, R. 1913 Über die Chemische Zusammensetzung einiger Pilze und über die bei der Autolyse derselben auftretenden Producte. *Landw. Vers. Sta.* 79/80: 541–562.

(134) YOSHIMURA, K., AND KANIA, M. 1913 Beträge zur Kenntnis der Stickstoffhaltigen Bestandteile des Pilzes *Cortinellus shiitake* P. Henn. *Ztschr. Physiol. Chem.* 86: 178–184.

(135) ZELLER, S. M. 1916 Studies in the physiology of the fungi. II. *Lenzites saepiaria*, Fries., with special reference to enzyme activity. *Ann. Missouri Bot. Gard.* 3: 439–512.

(136) ZELLNER, J. 1907 Chemie der höheren Pilze. Leipzig.

ym. of nitrogen from various sources.
ater-soluble and water-insoluble nitrogen

ater-soluble and water-insoluble nitrogen
1927).

FIG. 1

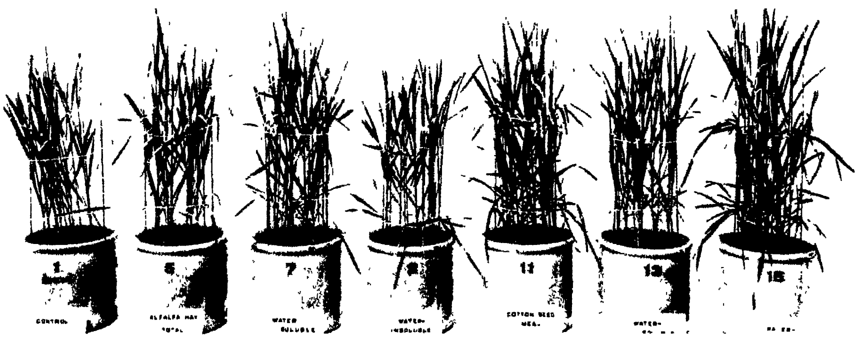


FIG. 2

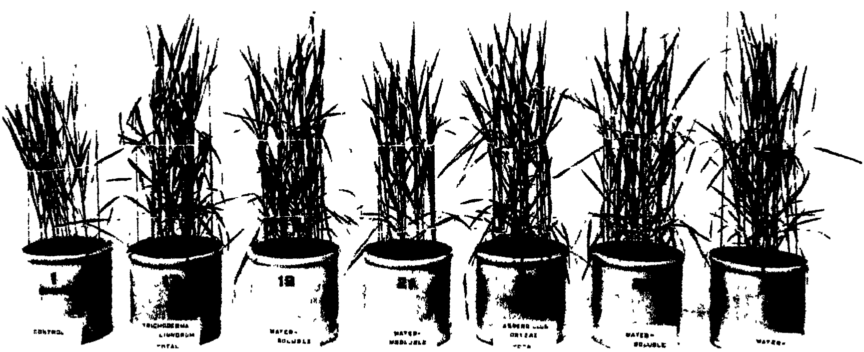


FIG. 3

THE INFLUENCE OF THE REPLACEABLE BASES ON THE SOIL SOLUTION FORMATION IN MINERALIZED SOILS

F. MENCHIKOWSKY AND S. RAVIKOVITCH

Palestine Zionist Executive Agricultural Experiment Station

Received for publication September 6, 1928

A considerable number of papers are devoted to the question of the formation and composition of the soil solution. In one of them, Whitney and Cameron (13) have reported that: "If every soil contains all the common rock forming minerals, every soil should give the same saturated solution."

The following investigations of the seasonal change in the soil solution composition (3, 11, 12) have shown, on the contrary, that the composition of the soil solution is in no way constant but that it changes in relation to the biochemical conditions of the soil and the growth of the plant.

On the basis of experiments carried out on leached soils, Burd (2) emphasized the importance of the biological processes for cation enrichment of the soil solution. On the grounds of these observations, Hoagland (8) defines the conditions of the soil solution formation in the following manner: "Normally a soil solution is in long measure a biologically controlled system; that is to say that nearly all the anion content of such a solution is of biological origin and equivalent quantities of cations must enter into solution with the anions."

The soil solution is composed of different parts. The salts of mineral origin which we detect in every soil, and chiefly in arid soils, may be dissolved at first if the soil contains a sufficient amount of water. The biochemical activity, on the other hand, as was pointed out in the foregoing, enriches the soil solution by anions and, in consequence of their action, also by cations taken from the insoluble portion of the soil. This source of the formation of the soil solution is very considerable, for the soils which have an important amount of organic matter are consequently characterized by their highly developed biological activity. As a source of the soluble parts of the soil solution we must take into account also the hydrolysis of the acid colloids of the soil, saturated with bases.

In the literature dealing with the question of soil zeolites some observations can be found about the conditions of the formation and decomposition of the zeolitic compounds with bases. Gedroiz (5) classifies the zeolites saturated with bases according to their stability in the following manner: Zeolites bound with CaO and MgO belong to the most durable compounds; those saturated with Na_2O and K_2O are less stable; the intermediate properties are shown by the zeolites saturated with H ion. These compounds of zeolites with bases are hydrolyzed by water with the appearance of free bases in the solution.

In the highly mineralized soils where organic matter is perhaps very insignificant, or where it is sometimes absent, the biochemical activity is consequently very low. The biological factor of the formation of the soil solution in those soils is therefore very limited. Although the influence of the slightly soluble minerals should not be important, the formation of the soil solution in such a case depends on the amount of easily soluble salts, but the chief factor here should be in the hydrolysis of the zeolitic compounds with bases.

The object of the experiments reported in this paper was to verify this view on the bases of some mineralized soils of Palestine.

SAMPLES OF SOIL FOR ANALYSIS

Three samples of soil from different parts of Palestine were chosen as material for study: 1. A sample of the heavy loamy soil from the coastal region in the vicinity of Ben-Shemen; 2. A sample of heavy loamy soil from the center of the Plain of Esdraelon south of Afule, from a place named Djuania; and 3. A sample of soil rich in lime from the Jordan Valley. The amount of organic matter in all these samples is insignificant, as is shown in table 1.

TABLE 1

The amount of organic matter in the soil samples

NAME OF LOCALITY	LAYER	PERCENTAGE OF DRY MATTER
	cm.	
Ben-Shemen	0– 25	0.81–0.78
Ben-Shemen	25– 50	0.61–0.59
Ben-Shemen	50– 75	0.54–0.52
Ben-Shemen	75–100	0.49–0.49
Djuania	0– 25	1.39–1.30
Djuania	25– 50	1.09–1.02
Djuania	50– 75	0.89–0.85
Djuania	75–100	0.65–0.65
Dagania	0– 25	1.22–1.19
Dagania	50– 75	0.56–0.53

The samples employed for the investigation were taken from four layers one below the other and each 25 cm. deep. In order to establish the relation existing between the replaceable bases of the soil and the soil extracts, the replaceable bases were studied in all the aforementioned samples.

The properties of the local heavy loamy soil make the preparation of the soil solution very difficult. In this work the water extract method was used. The experiments were carried out with 15, 25, 50, and 100 parts of soil to 100 parts of water. The comparison of the figures of the different water extracts makes it possible to determine the compounds which existed in the soil solution as distinct from those formed by hydrolysis.

THE METHODS

Methods have been proposed for the estimation of the amount of replaceable bases in a soil. The oldest method proposed by Gedroiz is based on the replacing of the absorbed bases by a solution of NH_4Cl; the second method recently recommended by him is the replacing with N 0.05 HCl (4). These methods, however, can not be applied in the presence of $CaCO_3$ in the soils, because both N 0.05 HCl and NH_4Cl dissolve $CaCO_3$. For the same reason, the method proposed by Bobko-Askenasi (1), based on the treating with $BaCl_2$, can not be applied. The most suitable method for Palestine soils rich in $CaCO_3$ is that of Hissink (7), based on the replacing of the absorbed Ca by the solution of NaCl. Hissink found that the quantity of $CaCO_3$ dissolved in one liter of NaCl is constant and that by a second and third treatment of the same soil with the solution of NaCl the further half liters of the NaCl solution show the same amount of dissolved $CaCO_3$. The value of this method was confirmed by the determinations of the absorbed Ca in our soils. The constant amount of the dissolved $CaCO_3$ in each second liter of NaCl solution after the soil has been treated should be observed. The figures are shown in table 2.

TABLE 2

The amount of CaO extracted from the soil by the second liter of the normal NaCl solution*

LOCALITY	LAYER	CaO IN 200 CC. N NaCl SOLUTION
	cm.	*gm.*
Ben-Shemen	0–25	0.0089
Ben-Shemen	25–50	0.0093
Ben-Shemen	50–75	0.0090
Ben-Shemen	75–100	0.0090
Djuania	0–25	0.0101
Djuania	25–50	0.0097
Djuania	50–75	0.0101
Djuania	75–100	0.0096
Dagania	0–25	0.0091
Dagania	25–50	0.0090

* $CaCO_3$ in the same soils: Ben-Shemen, 15.5 per cent; Djuania, 17.5 per cent; Dagania, 40.1 per cent.

The soil extracts were prepared from air-dry soil passed through the 2-mm. sieve and shaken with water for one hour. The liquid was filtered through the funnel without contact with the air. The paper for the filtration was washed several times with distilled water and was checked as to presence of soluble salts and traces of acid.

The following methods were used for the estimation of anions: Cl^- was determined by titration with 0.02 N $AgNO_3$; HCO_3^- with 0.1 N HCl in the presence of methyl orange; NO_3^- was estimated by the colorimetric method with phenol-sulfonic acid. Before the soil was tested, Cl^- was separated with

Ag_2SO_4. For $PO_4^{\equiv}$ the determination method of Deniges, modified by Shmuk and Kurilo (9) was employed. $SiO_3^{=}$ was also measured colorimetrically (10). The method is based on the color formed by ammonium molybdate. The several tests of the standard solution from K_2SiO_3 have shown that the boiling of the solution and the addition of the strong acid, lead to the partial coagulation of SiO_2. This method was therefore changed. To the solution was added diluted HNO_3 (d = 1.12, 1:4) and 5 per cent ammonium molybdate. The tested solution was not heated. The cations Ca, Mg, K, and Na were

TABLE 3

The amount of replaceable bases in the soils tested

ORIGIN OF A SAMPLE	MGM. EQUIVALENT IN 100 GM. DRY SOIL					PERCENTAGE OF A REPLACEABLE CATION TO THE TOTAL OF REPLACEABLE BASES IN 100 GM. DRY SOIL					
	Ca	Mg	K	Na	Total	Ca	Mg	K	Na	Ca + Mg	K + Na
The heavy loamy soil from Ben-Shemen											
cm.											
0– 25	41.9	17.2	0.85	6.73	66.68	62.8	25.8	1.3	10.1	88.6	11.4
25– 50	38.8	16.4	0.59	3.91	59.70	65.0	27.4	1.0	6.6	92.4	7.6
50– 75	36.4	15.5	0.36	7.62	59.88	60.8	25.8	0.6	12.8	86.6	13.4
75–100	33.5	18.5	0.26	11.97	64.23	52.2	28.8	0.4	18.6	81.0	19.0
The heavy loamy soil from Djuania											
cm.											
0– 25	33.1	19.9	1.27	3.78	58.05	57.0	34.3	2.2	6.5	91.3	8.7
25– 50	29.4	21.9	0.64	4.92	56.86	51.7	38.5	1.1	8.7	90.2	9.8
50– 75	25.9	22.5	0.41	5.04	53.85	48.1	41.8	0.7	9.4	89.9	10.1
75–100	24.1	22.7	0.35	7.64	54.79	44.0	41.4	0.6	14.0	85.4	14.6
Soil rich in lime from Jordan Valley (Dagania)											
cm.											
0 –25	26.6	5.47	0.41	1.50	33.98	78.3	16.1	1.2	4.4	94.4	5.6
25– 50	24.8	6.23	0.12	1.78	32.93	75.3	18.9	0.4	5.4	94.2	5.8
50– 75	21.5	7.57	....	2.62	31.69	67.8	23.9	...	8.3	91.7	8.3
75–100	18.3	10.31	....	2.34	30.95	59.1	33.3	...	7.6	92.4	7.6

measured by the common analytical method. All the calculations are made on dry matter.

REPLACEABLE BASES IN SOILS

The results of the study of the aforementioned soils concerning the bases absorbed by the colloidal part are reported in table 3. From the figures obtained it appears that these three soils possess a different quantity of replaceable bases. The heavy loamy soil of Ben-Shemen has the largest quantity

of replaceable bases, 64–67 mgm.—equivalents in 100 gm. of soil; the sample from the Plain of Esdrealon, 58–55; and the one from the Jordan Valley, 34–31.

As a rule the sum of the equivalents decreases in the direction of the lower layers, except in the fourth layer of Ben-Shemen and Djuania. The distribution of replaceable Ca, Mg, K, and Na in all these layers is very important. Ca, as we may conclude from the foregoing data, gradually decreases in all soils from the first layer to the fourth; Na, on the contrary, increases in the same direction. Thus the soils of Ben-Shemen form in a depth of more than 50 cm. a soil type similar to "Solonetz." According to its chemical properties Mg. resembles on one side the bivalent alkali earth metals and on the other side the alkali metals. Its properties have an influence also on its distribution among

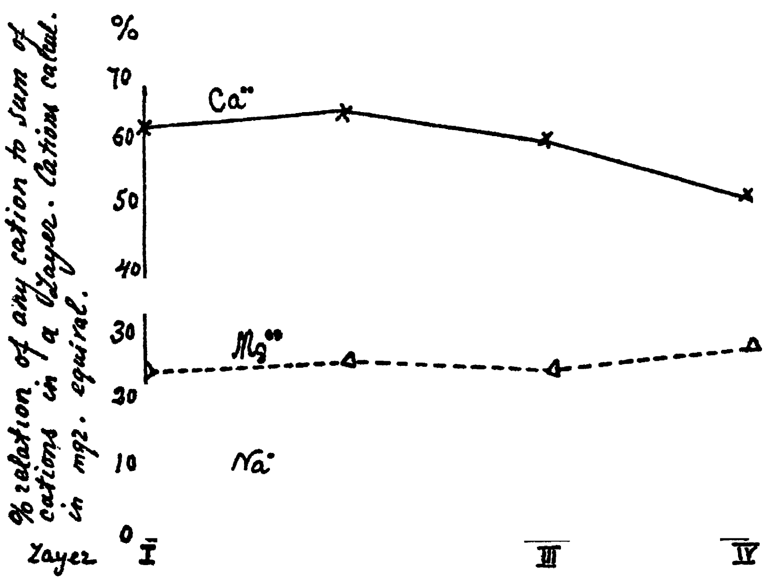


FIG. 1. DISTRIBUTION OF REPLACEABLE CATIONS IN BEN-SHEMEN SOIL

the layers of the soil. In the two samples (Ben-Shemen and Djuania) where replaceable Na is present in a large quantity, replaceable Mg is approximately constant, and only in the soil of the Jordan Valley does it increase in the lower layers. In this soil Mg seems to replace Na, which is present here in a small quantity.

Base exchange phenomena appear also in the natural conditions of the soil. The law of mass action, the quantity of any dissolved cation which exists in the soil in the form of any salt, and the replacing activity of the cation are the chief factors which regulate these phenomena. The quantity of the replaceable bases and their distribution among the layers of the soil can be observed as a result of an established equilibrium. The graphical interpretation of our figures of the replaceable bases in milligram-equivalents in the layers, illustrates

sting relation between different replaceable cations. Figure 1 repre- ıese relations for the Ben-Shemen soil. The shape of the curves for Ca ; is remarkable. The state of saturation of the zeolites by Ca in each : the soil is inverse to the Na. This can be explained as a result of the action between $CaH_2(CO_3)_2$ and NaCl in the soil solution and the zeoli- ıplex.

result of relatively much higher biochemical activity, Ca being in- l by the HCO_3^- and NO_3^- formed, dissolves much more completely in ıtion of the upper layers of the soil. On the other hand NaCl, which is 'ely leached out as a very soluble salt from the upper layers, accumu-

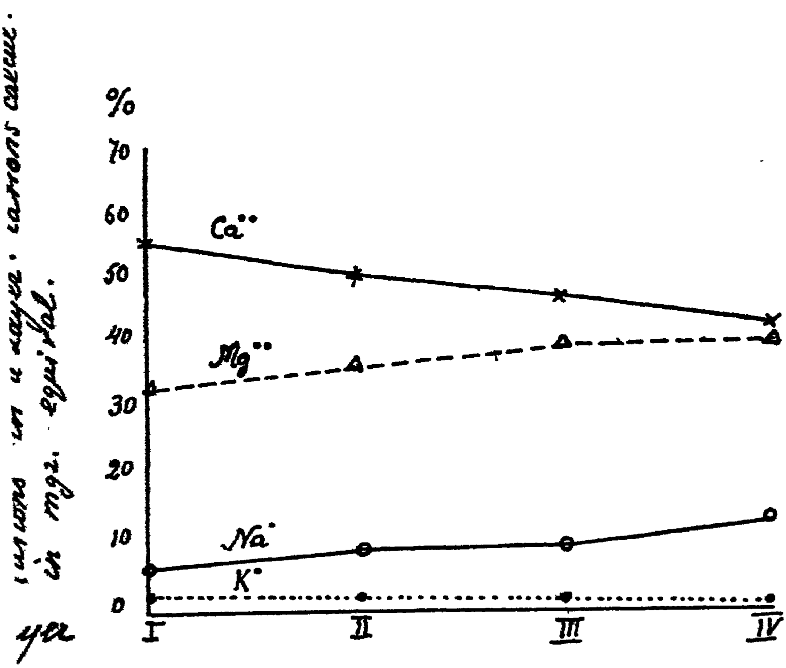


FIG. 2. DISTRIBUTION OF REPLACEABLE CATIONS IN DJUANIA SOIL

he lower layers. This leads to a saturation of the upper layers by Ca. ontrary, the increasing quantity of NaCl in the soil solution of the ers influences the enrichment of the zeolite by Na. A similar relation bserved in the soil of Djuania (fig. 2) where the curves of Ca and Na nverse to each other.

soil of Ben-Shemen, Mg does not remain constant, but increases ıately in a parallel line to replaceable Na. Quite another relation bserved between the replaceable cations in the soil of the Jordan)agania). The inverse relation applies here to the curves of Ca and 3). In the soil from Dagania, the easily dissolved salts are present in

very small quantities. In connection with this circumstance the colloid soil complex must be very slightly saturated by Na and K. The figures obtained for the replaceable Na and K in the investigated sample of the Dagania soil are very small when compared with the figures of Na and K found for Ben-Shemen and Djuania soils. The cation K is absolutely absent in the third and fourth layers. This is probably the reason why Mg appears as a cation replacing Ca in the lower layers. $MgCO_3$ is usually present in the calcareous soils together with $CaCO_3$ and the solubility of $MgCO_3$ is many times greater than that of $CaCO_3$. This is why $MgCO_3$, in the absence of any appreciable quantity of NaCl, appears in the lower layers as a salt displacing Ca and re-

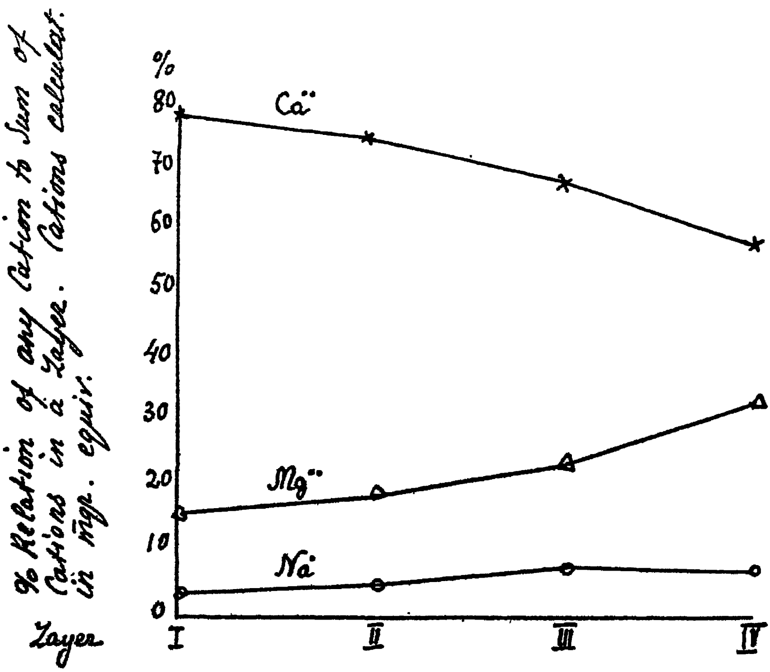


FIG. 3. DISTRIBUTION OF REPLACEABLE CATIONS IN DAGANIA SOIL

placing it in the colloid complex. When the soil possesses an important quantity of NaCl, Mg is usually replaced by Na.

The curves of the distribution of the replaceable bases which are present in the colloidal part of the soil in its different layers can be easily explained as a result of equilibrium between the soil and the cations taking part in the base exchange.

DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF SOIL

The supposition that the state of saturation of the colloidal complex of the soil is the result of the action of different cations which for a certain time are in

contact with the soil, leads the authors to admit the reverse phenomenon—the transition of the cations from the complex to the soil solution, i.e. if, for one reason or another, the solution will become poor in cations.

The decomposition pressure in the water medium of the soil zeolites saturated by different cations can not be the same. Apart from the properties of

TABLE 4

Water extracts from soil of Ben-Shemen

Ratio of soil to water 15:100

DEPTH	Ca	Mg	Na	NH_4	PO_4	SO_4	HCO_3	NO_3	Cl	SiO_3	TOTAL
cm.											
Amount of ions extracted from 100 gm. of dry soil, in mgm.											
0– 25	25.6	6.0	6.9	......	Trace	......	48.7	0.83	11.5	6.8	
25– 50	17.6	5.7	6.0	......	....	......	35.0	0.52	11.7	5.5	
50– 75	13.4	5.2	19.1	......	....	......	44.0	0.45	11.7	6.0	
75–100	15.0	6.8	30.1	......	....	......	58.0	0.34	20.7	6.0	
Amount of ions extracted from 100 gm. of dry soil, in mgm. equivalent											
0– 25	1.28	0.50	0.30	......	....	......	1.57	0.013	0.32	0.18	4.16
25– 50	0.88	0.47	0.26	......	....	......	1.13	0.009	0.32	0.14	3.22
50– 75	0.67	0.42	0.83	......	....	......	1.42	0.007	0.32	0.16	3.84
75–100	0.75	0.56	1.31	......	....	......	1.87	0.005	0.58	0.16	5.24

TABLE 5

Water extracts from soil of Djuania

Ratio of soil to water 15:100

DEPTH	Ca	Mg	Na	NH_4	PO_4	SO_4	HCO_3	NO_3	Cl	SiO_3	TOTAL
cm.											
Amount of ions extracted from 100 gm. of dry soil, in mgm.											
0– 25	14.0	6.8	6.9	......	Trace	......	35.9	0.71	5.1	9.6	
25– 50	9.3	5.5	20.0	......	....	......	42.5	0.39	4.1	10.5	
50– 75	6.6	3.2	29.9	......	....	......	43.3	0.30	5.9	11.7	
75–100	6.1	2.8	43.2	......	....	......	57.6	0.33	5.9	14.0	
Amount of ions extracted from 100 gm. of dry soil, in mgm. equivalents											
0– 25	0.70	0.56	0.30	......	....	......	1.16	0.012	0.14	0.25	3.12
25– 50	0.46	0.45	0.87	......	....	......	1.37	0.006	0.12	0.28	3.56
50– 75	0.33	0.26	1.30	......	....	......	1.40	0.005	0.17	0.31	3.78
75–100	0.30	0.23	1.88	......	....	......	1.86	0.005	0.17	0.37	4.82

the cations forming with the acid soil complex more or less stable compounds, the phenomena observed in the soil lead us to assume the presence of fractions of decreasing acidity which when saturated by the same cation may form com-

pounds of different stability. As a result of the kind of cation and of the saturation of the more or less acid "acidoides" of the soil the latter may contain a series of compounds of different composition and solidity which, when the soil is treated with water, forms a soil solution. The composition and the concentration of the cations in the solution reflect the state of saturation of the soil colloids by the bases. It is possible that a part of the cations of the relatively concentrated soil solution are formed by the hydrolysis of zeolitic compounds of the soil, but in order to obtain a more evident idea of the formation of the soil solution from the bases absorbed by the soil zeolites, the authors deemed it necessary to investigate more diluted water extracts of the soil, where naturally the hydrolysis occurs very intensively.

TABLE 6

Water extracts from soil of Dagania

Ratio of soil to water 15:100

DEPTH	Ca	Mg	Na	NH_4	PO_4	SO_4	HCO_3	NO_3	Cl	SiO_2	TOTAL
cm.											
Amount of ions extracted from 100 gm. of dry soil, in mgm.											
0– 25	14.5	4.5	3.5	Trace	......	....	27.9	0.42	1.7	10.6	
25– 50	13.2	4.5	1.8	....	......	....	25.6	0.34	2.1	7.5	
50– 75	11.6	4.7	....	....	......	Trace	23.2	0.17	2.1	6.0	
75–100	11.2	5.8	....	....	......	3.2	23.2	0.22	2.3	6.0	
Amount of ions extracted from 100 gm. of dry soil, in mgm. equivalent											
0– 25	0.72	0.37	0.15	....	......	....	0.90	0.007	0.05	0.28	2.48
25– 50	0.65	0.37	0.08	....	......	....	0.83	0.006	0.06	0.20	2.20
50– 75	0.58	0.39	....	....	......	....	0.75	0.003	0.06	0.16	1.94
75–100	0.56	0.48	....	....	......	0.07	0.75	0.004	0.07	0.16	2.09

Extracts of 15 gm. of soil to 100 cc. of water were prepared for the following examinations. The results are represented in tables 4, 5, 6. The figures found for each cation expressing in per cent the total amount of cations for each layer of the soil of Ben-Shemen, Djuania and of Dagania are illustrated in figures of 4, 5, 6. When these curves are examined and compared with figures 1, 2, and 3 showing the distribution of the replaceable bases, some relations can be observed which bear the character of a law. We have observed before that the curves of the replaceable cations Ca and Na in the different layers of the Ben-Shemen soil are inverse to one another. The resembling draft of the curves for Ca and Na can be observed in the soil extract. The same relations can be seen by comparing the curves of the soil and the soil extract of the samples of Djuania and Dagania. *We have therefore some reason to believe that the distribution of cations of the water extract of the soils in a certain degree reflects the distribution of the same cations present as replaceable in the*

colloid complex of the soil. From the curves of figures 4, 5, and 6 further conclusions can be drawn. In the case of the soils highly saturated with Na this inverse character of the curves appears more evident than in the case of soils with a small quantity of absorbed Na. This phenomenon is caused by the character of the complex exposed to hydrolysis. As the observations show, the colloidal soil complex changes in relation to size and composition. The colloidal part of the soil which is the main bearer of the absorbing properties of the soil can be easily disintegrated and under certain conditions taken out from the soil both in a changed and an unchanged state. The water acting in a disintegrating and decomposing manner on the salt-like compounds of the

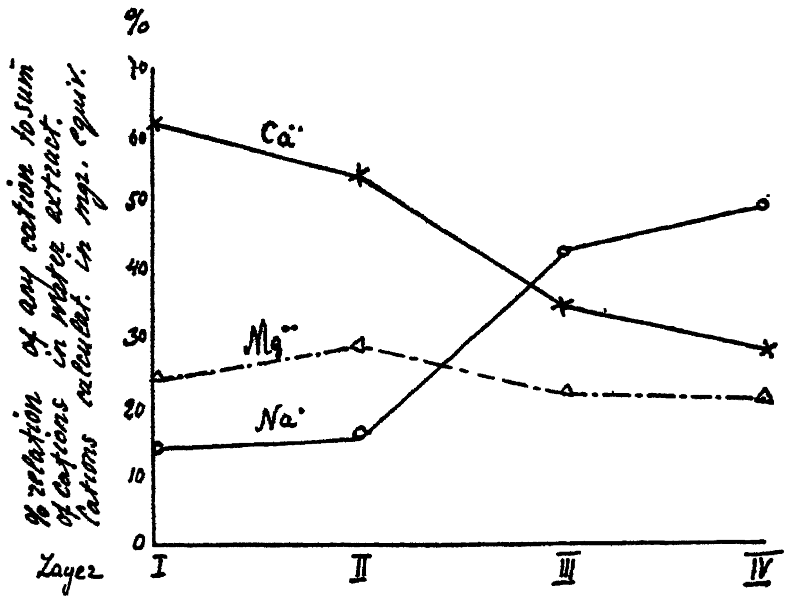


FIG. 4. DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF BEN SHEMEN SOIL

Ratio of Soil to Water 15:100

colloidal complex and on the split alumino-silicate nucleus is the cause of these phenomena. The parts of the complex highly saturated by Na are at first exposed to destruction because they belong to the less solid and easily hydrated, disintegrated, and hydrolyzed soil compounds. The soil complex exposed to intensive leaching not only loses its Na very rapidly but the soil groups which were bound with Na also submit to disintegration. The size of the colloidal absorbing complex generally decreases as a result of leaching and the parts more sensible to hydrolysis diminish too.

The soils of Dagania belong to the type of soils possessing a very low absorbing complex (S = 34 mgm. equivalent in 100 gm. of soil), and the presence

of only traces of dissolved salts demonstrates the strong leaching of these soil types. The remaining part of the soil absorbing complex is resistant to water and being also saturated with Ca and Mg gives very solid and slightly hydrolyzed compounds. For this reason the cation curves of the soil extract of Dagania are only slightly different from the curves of the replaceable cations in the same soil.

The colloidal soil complexes of Ben-Shemen and Djuania are in quite different conditions. The absorbing complex of these soils is very high ($S = 67$ and $S = 58$ mgm. equivalents in 100 gm. of soil) and the great part of it consists of

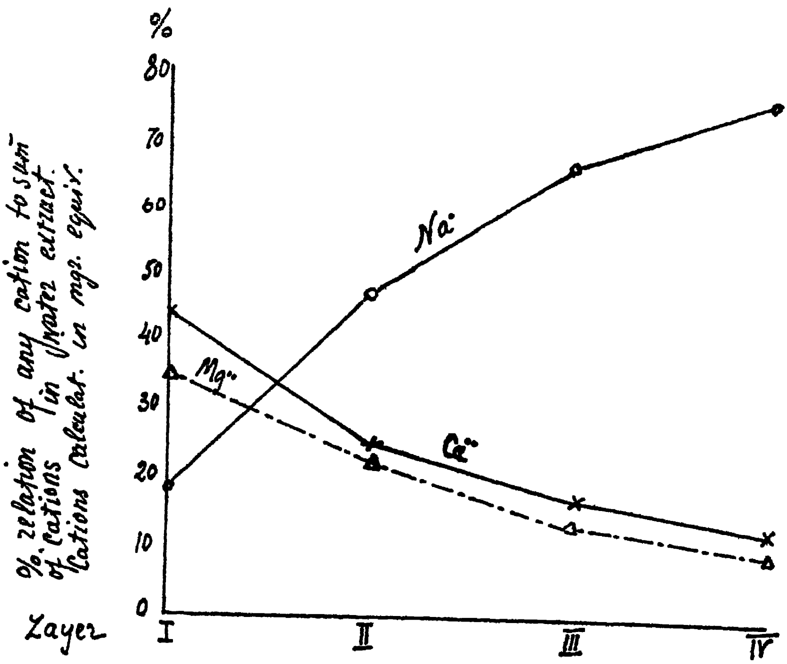


FIG. 5. DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF DJUANIA SOIL

Ratio of Soil to Water 15:100

unstable, easily hydrolyzed compounds. As a result of the water action, these soils are exposed to very strong hydrolysis and therefore the inverse character of the curves drawn for the cations of the water extract from the Djuania and Ben-Shemen soils is more conspicuous in comparison with the curves for the replaceable bases of the same soils.

Gedroiz (5) explains, by the properties of the replaceable cation, the degree of stability of the compound formed by the acid-absorbing part of the soil with the base. This property depends not only on the cation but also on the acid portion with which the cation is bound. It is very probable that there

exist in the soil (in this particular case in the mineral part of the soil) acid groups which form with the same cation some compounds of different stability. The saturation of the soil with a base takes place gradually. At first the very acid groups of the soil become saturated and form very solid compounds. By subsequent saturation with a base, less acid groups come into action and form with the bases less solid substances which readily decompose. If the soil contains some cations of different reactive power, then competition takes place at first for the formation of the compounds with the less acid groups where the cation is bound less strongly, and only later for the more solidly fixed cations.

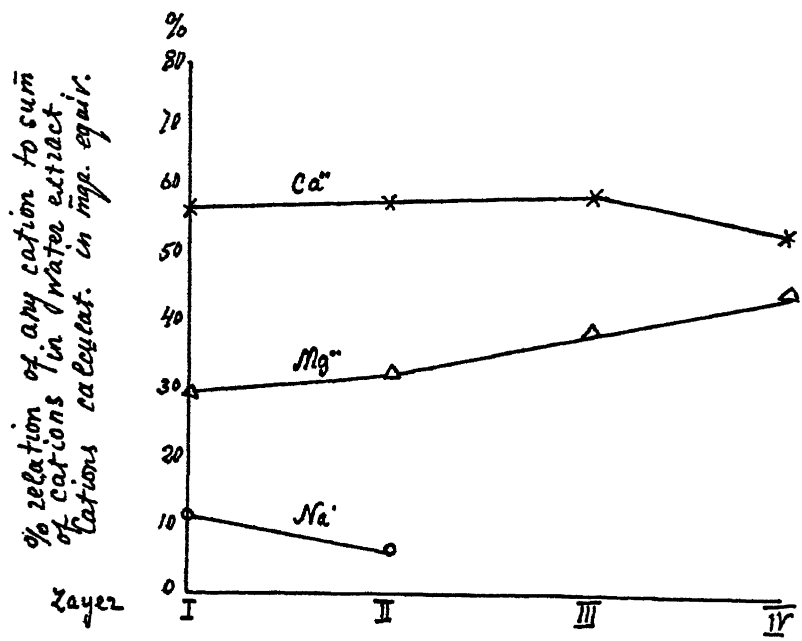


FIG. 6. DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF DAGANIA SOIL

Ratio of Soil to Water 15:100

In the case of hydrolysis, the first to decompose are the unstable compounds, formed with the cations during the highest state of saturation. This means, as we may expect, that the higher the amount of any replaceable cation in a colloidal complex the stronger the degree of hydrolysis.

Figure 7 shows the relation between the degree of hydrolysis and the amount of the cation in the absorbing complex of the soil. The amount in milligram-equivalents of a cation as a percentage of the total amount of replaceable bases in the soil sample is represented on the abscissa and the percentage of the quantity of milligram-equivalent of the same cation extracted from 100 gm. of soil to the total amount of replaceable bases in 100 gm. soil is represented on the

ordinate. The curves are plotted for Na and Ca for the soils of Djuania and Ben-Shemen. The curves show a high hydrolysis for Na in comparison to Ca, which agrees with the literature. According to Gedroiz (5) the replacing energy of different cations decreases in the following order: Ca, Mg, K, Na, the degree of hydrolysis of the replaceable bases being inverse for the same cations.

Another feature of these curves is the increasing degree of hydrolysis with an increase in the degree of saturation of the soil colloidal complex. This is more

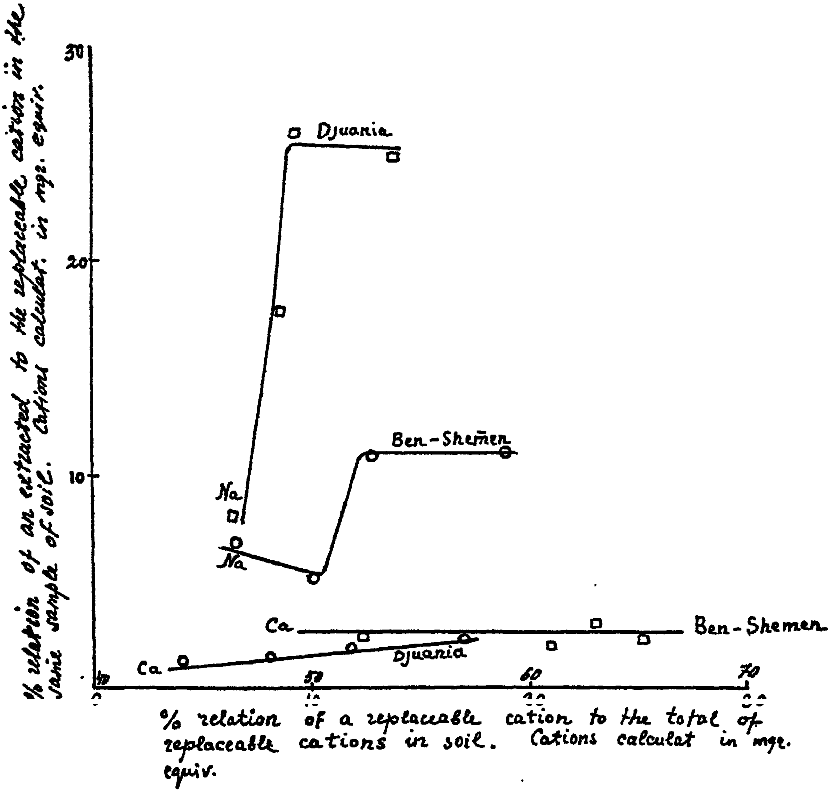


FIG. 7. HYDROLYSIS OF REPLACEABLE CA AND NA IN SOILS FROM BEN-SHEMEN AND DJUANIA

Ratio of Soil to Water 15:100

conspicuous for Na than for Ca. The results obtained support the theories of the author about the presence of some acid groups of different stability in the soil and of the formation of compounds of decreasing stability according to the increasing saturation of the colloidal soil complex by a cation.

INFLUENCE OF THE PROPORTION OF SOIL TO WATER ON THE COMPOSITION OF ANIONS AND CATIONS IN THE SOIL EXTRACT

In order to learn whether the aforementioned results for the soil extract can be applied to the much more concentrated soil extracts a series of water

TABLE 7

Amount of cations and anions in mgm. leached out from 100 gm. soil in different proportions of soil to water

Sample of Djuania soil. Calculated on dry matter

DEPTH	Ca				Mg				Na			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50.100	25:100	15:100
cm.												
0–25	4.0	7.3	11.6	14.0	1.9	2.9	5.0	6.8	1.8	3.7	6.4	6.9
25–50	2.4	4.4	6.3	9.3	1.5	2.5	3.5	5.5	5.1	8.5	13.6	20.0
50–75	...	2.6	4.7	6.6	...	1.7	2.3	3.2	...	12.0	18.9	29.9
75–100	...	2.1	3.9	6.1	...	1.5	1.8	2.8	...	16.1	25.8	43.2

DEPTH	HCO_3				Cl				SiO_3				NO_3			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cm.																
0–25	8.3	16.6	29.0	35.9	4.1	4.2	4.6	5.1	1.4	3.5	7.3	9.6	0.78	0.75	0.71	0.71
25–50	10.3	18.6	27.7	42.5	2.9	3.0	3.5	4.1	1.6	4.1	7.2	10.5	0.34	0.36	0.40	0.39
50–75	....	17.6	28.6	43.3	....	4.1	4.8	5.9	...	3.7	6.5	11.7	...	0.22	0.28	0.30
75–100	....	21.0	35.2	57.6	...	4.8	4.9	5.9	...	4.0	6.6	14.0	...	0.28	0.29	0.33

Amount of cations and anions in mgm. equivalents leached out from 100 gm. soil in different proportions of soil to water

DEPTH	Ca				Mg				Na				Total in mgm. equivalents			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cm.																
0– 25	0.20	0.36	0.58	0.70	0.16	0.24	0.41	0.56	0.08	0.16	0.28	0.30	0.88	1.52	2.54	3.12
25– 50	0.12	0.22	0.31	0.46	0.12	0.21	0.29	0.45	0.22	0.37	0.59	0.87	0.92	1.60	2.38	3.56
50– 75	....	0.13	0.23	0.33	....	0.14	0.19	0.26	....	0.52	0.82	1.30	....	1.58	2.48	3.78
75–100	....	0.11	0.19	0.30	....	0.12	0.15	0.23	....	0.70	1.12	1.88	....	1.86	2.92	4.82

DEPTH	HCO_3				Cl				SiO_3				NO_3			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cm.																
0– 25	0.27	0.54	0.94	1.16	0.12	0.12	0.13	0.14	0.04	0.09	0.19	0.25	0.013	0.012	0.012	0.012
25– 50	0.33	0.60	0.89	1.37	0.08	0.08	0.10	0.12	0.04	0.11	0.19	0.28	0.006	0.006	0.007	0.006
50– 75	....	0.57	0.92	1.40	....	0.12	0.14	0.17	....	0.10	0.17	0.31	....	0.004	0.005	0.005
75–100	....	0.68	1.14	1.86	....	0.14	0.14	0.17	....	0.10	0.17	0.37	....	0.005	0.005	0.005

Ions NH_4, SO_4, PO_4 absent.

extracts with an increasing proportion of soil to water were analyzed. Table 7 shows the numerical results of the analysis made with the water extracts of Djuania soil in the proportions 15, 25, 50, and 100 parts of soil to 100 parts of water. The figures in this table give valuable data for judging the origin of anions and cations of which the soil extract was formed. Some interesting properties may be observed in the anion part of extracts obtained from the investigated soil. First, it is remarkable that PO_4 and SO_4 are absent among the anions except in the fourth layer of Dagania soil (see tables 4, 5, and 6). Cl and NO_3 occupy a particular place between other anions. It is to be expected that salt present in soluble form in the soil will show by dilution with water that its concentration is inverse to the degree of dilution. The amount of milligram equivalents extracted from the same quantity of soil remains unchanged in all proportions of soil to water. From the numeral data concerning nitrates it may be observed that in all proportions of soil to water the quantity of NO_3 expressed in milligram-equivalents is unchangeable. From this it is evident that NO_3^- is a part of a salt present in a soluble form in the soil. As to Cl^- we can say that the salt of this anion is to some extent absorbed by the soil, although in its general properties it resembles the salt dissolved in water.

The two other anions of the soil extract—HCO_3^- and $SiO_3^=$—possess quite different properties. The quantity of both of these anions increases, in the extract from the same quantity of soil, with the increasing proportion of water to soil. In the extracts of our soils which are poor in organic matter we can not attribute the origin of HCO_3^- to CO_2. It would be more correct to suppose that simultaneously with the appearance of the bases in the solution after hydrolysis CO_2 is derived from the air and leads to the formation of HCO_3^- in the extract. The ion of $SiO_3^=$, the quantity of which also increases together with the increase of the proportion of water to soil, is evidently caused by the decomposition of some silicic soil compounds.

In soils rich in organic matter the methods of investigation of soil extracts render difficult any attempt to interpret the following question: "Does water in fact destroy the alumino-silicate nucleus of the primary particles of the colloidal complex or does it eject the fine powdered particles of the complex?" (7), even to make clear the question about the part played by the replaceable cations in these processes.

Our observations on the amount of $SiO_3^=$ in the soil extract obtained by treating the soil samples with water show as a general rule that the dissolved $SiO_3^=$ increases with the increase of the amount of water added to the soil and consequently there takes place a disintegration of the alumino-silicate particles. Should the opinions on the durability of the zeolitic nucleus dependent on replaceable bases prove to be accurate, we might expect the decomposition of the alumino-silicate nucleus in the lower layers of the soil where it is saturated with Na. This viewpoint would correspond to the well-known properties of replaceable Na, which intensively disintegrate the colloidal complex of the soil.

With regard to the relation which may probably exist between $SiO_3^{=}$ formed in a water extract and the replaceable Na the following facts can be observed in our data. In water extracts from the Ben-Shemen soils the amount of $SiO_3^{=}$ may be considered in all layers as a constant, without taking into account the unavoidable error of our method. In water extracts from the Djuania soil (table 7) where the proportions of the soil to water are 100, 50, and 25 to 100, one may assume the same amount of SiO_3 in all layers. An exception to the rule is in the two upper layers of the Dagania soil where we can observe an increase of $SiO_3^{=}$ in the water extracts.

On the basis of our data we can conclude from the continually increasing amount of replaceable Na in all investigated soils, that $SiO_3^{=}$ appears to be constant many times in the soil extracts and is only sometimes on the decrease. It follows that the appearance of this anion in water extracts is not dependent on the replaceable Na.

TABLE 8

Percentage of each cation in relation to total amount of cations extracted from 100 gm. soil

Taken for any layer in different proportions of soil to water

DEPTH	Ca				Mg				Na			
	Proportion of soil to water				Proportion of soil to water				Proportion of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cm.												
0–25	45.5	47.3	45.7	44.9	36.3	31.6	32.3	35.9	18.2	21.1	22.0	19.2
25–50	26.1	27.5	26.0	25.8	26.1	26.2	24.4	25.3	47.8	46.3	49.6	48.9
50–75	....	16.5	18.6	17.5	....	17.7	15.3	14.3	....	65.8	66.1	68.2
75–100	....	11.8	13.0	12.5	....	12.9	10.3	9.5	....	75.3	76.7	78.0

It has been shown above in the samples of soils from Ben-Shemen, Djuania, and Dagania that the soil extract depends for its composition on the replaceable bases. The conclusions were based on the observations made on weak water extracts and it might be expected that with the increase of the concentration of the soil extract these relations would be different.

The following results were obtained by studying the water extract in various proportions of soil to water. By summing up the cations and anions of the soil extract we find that the total amount, expressed in milligram equivalents extracted from 100 gm. of soil, simultaneously increases with the proportion of water to soil, i.e. because of the influence of the water, a part of the insoluble compounds of the soil enters the solution. This process of transition of insoluble ingredients into the soil extract proves to be regular. If each cation is expressed in the percentage of the total amount of cations in the water extract in certain proportion of soil to water for any soil layer the percentage of each cation in the soil extract remains unchanged and independent of the general

increase of cations during the dilution. These calculations are reported in table 8 and the same relations are graphically interpreted in figure 8. *The distribution of the cations in a soil extract is independent of the degree of dilution*

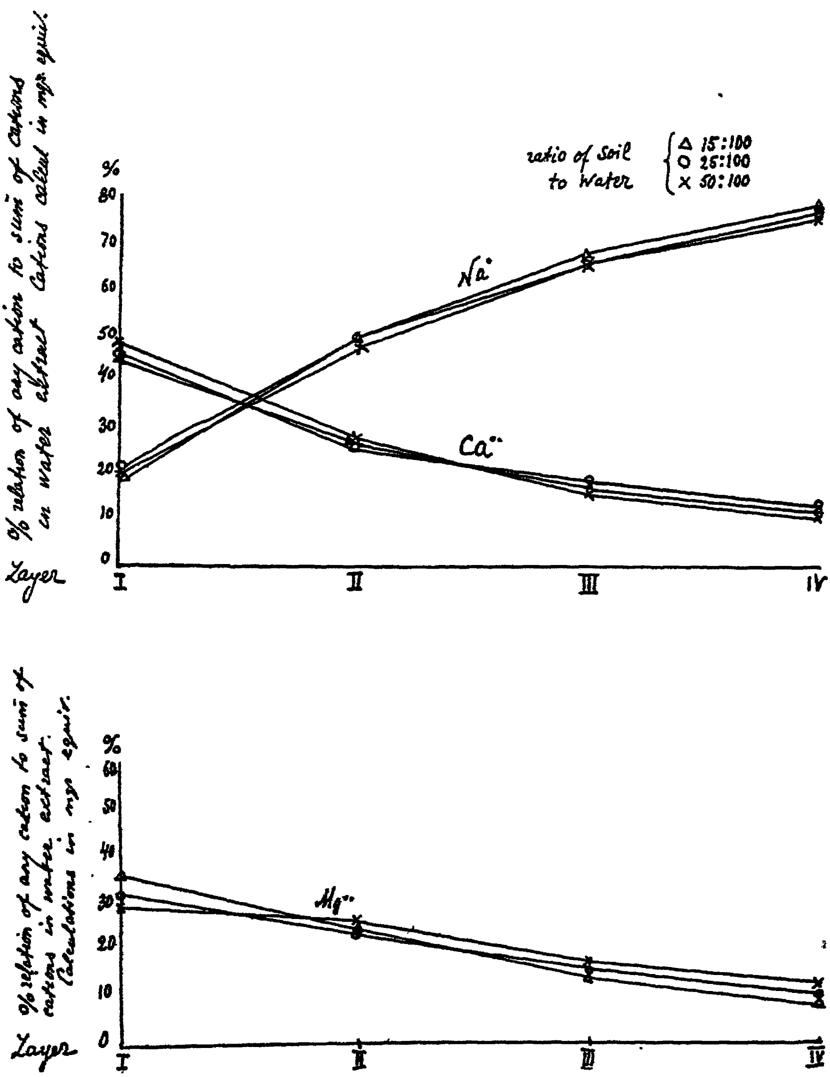


FIG. 8. THE INFLUENCE OF THE PROPORTION OF SOIL TO WATER ON THE DISTRIBUTION OF CATIONS IN DJUANIA SOIL EXTRACT

of soil in water and the appearance o each cation in solution strictly corresponds to the partial decomposition pressure of the compounds formed by replaceable cations and the zeolitic nucleus.

The results of our observations on water extracts of the soil lead to conclusions on the composition of the soil solution. It was found, as stated in the foregoing, that in water extracts of different proportions of soil to water the general amount of the extracted anions Cl^- and NO_3^- remains constant, but that HCO_3^-, on the contrary, increases with the increase of the proportion of water to soil. Hibbard (14) compared the composition of the soil solution obtained by the displacement method with the composition of the soil extract and drew the same conclusions in regard to the Cl, NO_3, and HCO_3 anions. We can thus establish the state of Cl^- and NO_3^- as parts of dissolved compounds in the fluid part of the soil but we may also decide on the grounds of the analysis of the soil extract about the origin of some other constituents of the soil solution. The constancy of the distribution of the cations in a soil extract both in low and high proportions of water to soil, permits us to draw conclusion also about the probability of the existence of this condition in the soil solution, i.e. in the relation of water to soil corresponding to the normal condition of soil moisture. It may be very probable that the soil solution of mineralized soils in its content of different cations and their distribution, reflects the state of saturation of the soil colloidal complex and its property of hydrolyzing under the influence of water.

SUMMARY

1. A study of the replaceable bases in three samples of soil from Ben-Shemen (Plain of Sharon), Djuania (Plain of Esdrealon), and Dagania (Valley of Jordan) demonstrated that the distribution of Ca and Na replaced from the first and second soil samples and of Ca and Mg from the third samples is inversed.

2. An examination of the water extracts of the soil (in the proportion of 15 gm. soil to 100 gm. water) of the aforementioned samples showed that the distribution curves of the corresponding cations in the extract according to the various soil layers conform to the distribution of replaceable bases of the soils. These curves, which are the results of the hydrolysis phenomena, are more distinctly expressed in the case of soils possessing large absorbing complexes.

3. An examination was made of a series of extracts from soils in which the proportion of soil to water was increased. It was found that, with the exception of one layer of the Dagania soil, the anions $PO_4^{\equiv}$ and $SO_4^{=}$ were absent in the soil extract. The amount of HCO_3^- and $SiO_3^{=}$ extracted from the soil was found to increase with dilution. Our observations show: (*a*) that the influence of the water leads to the decomposition of the alumino-silicate nucleus of the colloidal complex, and (*b*) that this decomposition does not depend on absorbed Na.

4. No_3^- and Cl^- bear the character of the anions of soluble salts. This corresponds to Hibbard's results.

5. The distribution of the cations in the soil extracts is not dependent on the degree of the increase of water in the soil. *The cations are distributed in the*

same percentage in all extracts, and the appearance of each cation in the extract corresponds to the partial decomposition pressure of the compounds formed by the absorbed cation with the alumino-silicate group.

6. Observations lead to the conclusion that in highly mineralized soils the composition of the soil solution relatively conforms with the composition of the replaceable bases in the soil colloidal complex, and to a certain degree reflects also the character of the same complex.

REFERENCES

(1) BOBKO, E. W., AND ASKINASI, D. L. 1925 Bestimmung der Adsorptionkapasität und des Ungesättigkeitsgrades der Böden. *Ztschr. Pflanzenernähr. u. Düngung* 6: 99–127.

(2) BURD, J. S. 1925 Relation of biological processes to cation concentration in the soil. *Soil Sci.* 20: 269–283.

(3) BURD, J. S., AND MARTIN, J. C. 1924 Secular and seasonal changes in the soil solution. *Soil Sci.* 18: 157–167.

(4) GEDROIZ, K. K. 1923 The hydrochloric acid method for determining in the soil the cations present in adsorbed condition. *Soil Sci.* 16: 473.

(5) GEDROIZ, K. K. 1927 Der adsorbierende Bodenkomplex, ed. 2. Nosowka Agr. Exp. Sta.

(6) HIBBARD, P. 1923 Comparison of soil solution by displacement method and water extract. *Soil Sci.* 16: 165–171.

(7) HISSINK, D. J. 1922 Beitrag zur Kenntnis der Adsorptionsvorgängim Boden. *Internatl. Mitt. Bodenk.* 12: 81–171.

(8) HOAGLAND, D. R. 1925 Physiological aspects of soil solution investigations. *Hilgardia* 1: 227–257.

(9) SHMUK, A., AND KURILO, M. 1926 *Jour. Landw. Wiss. (Moskau)* 3.

(10) SNELL, F. D. 1921 Colorimetric Analysis. New York.

(11) SOBOLEW, F., AND DRATSCHEW, S. 1926 Ueber die Wirkung von Bearbeitung und Düngung auf die Dynamik der Bodenlösung und der absorbierten Kationen im Boden. *Jour. Landw. Wiss. (Moskau)* 2: 97.

(12) STEWART, G. R. 1918 Effect of season and crop growth in modifying the soil extract. *Jour. Agr. Res.* 12: 311–368.

(13) WHITNEY, M., AND CAMERON, F. K. 1903 The chemistry of the soil as related to crop production. U. S. Dept. Agr. Bur. Soils Bul. 22.

(14) WIEGNER, G., GALLAY, R., AND GESSNER, H. 1924 Wasserbindung im Boden. *Kolloid Ztschr.* 35: 313–322.

THE TOLERANCE LIMIT OF SEEDLINGS FOR ALUMINUM AND IRON AND THE ANTAGONISM OF CALCIUM

JOHN R. SKEEN

University of Pennsylvania

Received for publication September 21, 1928

That the presence of Al in the soil is a factor in the distribution of plants was first shown by Hartwell and Pember (7). The work of many other investigators has established the fact. Blair and Prince (3) show that the toxic effect of a soil may be due to the presence of Al and Fe while entirely independent of pH. Quoting from a later work of these authors (4) ". . . . enough work has been done to show that acids decrease pH and increase the amount of active Al in the soil."

That the only significant part of the soil with respect to plant nutrition is the soil solution, is well established. Wilting point determinations show that only a fraction of the capillary water is available for plant use; further, cell walls of roots and root hairs are many times thicker than the hygroscopic water film immediately surrounding the soil particles. In a complex colloid system as presented by soils, a prediction of the equilibrium established in the solution from total analysis data is extremely hazardous. Blair and Prince (3) demonstrated the benefit to be derived by "heavy application of acid phosphate to toxic soils" and Magistad (15) indicates the solubility of Al with respect to falling pH and the presence of phosphates.

From total phosphate determinations and pH of the soil, together with p.p.m. Fe and Al in the soil solution, the writer attempted to account for the quality of five soils obtained in Berks and Montgomery Counties in the summer of 1926. Three of the soils were unquestioned "bad producers" and two, used as checks, were said to be excellent. The pH's of the latter were 5.7 and 6.6 whereas those of the former were uniformly low, from 4.3 to 4.6. Determinations showed no significant differences in total phosphate of the five soils, whereas Fe and Al were present in from 0.1 to 1.5 p.p.m. in all cases. That the toxicity of free acid does not explain soil quality has been demonstrated in a previous paper (16) wherein the important rôle played by Ca has been suggested.

It is the object of this paper to establish the limits of Al and Fe concentration that can be tolerated by two type plants, *Lupinus albus* and *Phaseolus vulgaris nanus* and to demonstrate the antagonistic action of Ca for these toxic elements.

The method used is essentially that reported in a preceding paper (16). The growth of the primary radical in millimeters is reported at intervals of 24

hours and the solutions into which the seedlings were put were changed every day. Average temperatures were determined by means of a thermograph.

RESULTS

Table 1 shows the toxic effect of Fe and the antagonism of Ca for Phaseolus. Three separate experiments were performed but the results of only one are presented. The tendency was the same in all cases.

TABLE 1

Growth of radicals of Phaseolus with various treatments of Fe and Ca at an average temperature of 18°C.

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	SIDE ROOTS
		mm.	*mm.*	*mm.*	
Water		7	8	8.2	Few and short
	p.p.m.				
Fe	0.05	8.3	9	9.5	Many blunt
	0.11	8	8.6	9.0	Many blunt
	0.23	7.7	7.7	7.0	Few blunt
	0.35	8.5	9.8	Dead	Few blunt
	p.p.m.				
Ca	5.0	13	22	28	Numerous, medium
	+ Fe 0.05	16	28	38	Numerous, medium
	+ 0.11	13	20	23	Few, medium
	+ 0.23	13	22	29	Few, blunt
	+ 0.35	12	19	24	Few, blunt
	p.p.m.				
Ca	10.0	17	31	45	Numerous, long
	+ Fe 0.05	16	31	43	Numerous, long
	+ 0.11	17	31	43	Numerous, long
	+ 0.23	18	35	51	Few, medium
	+ 0.35	20	43	63	Few short
	p.p.m.				
Ca	33.0	19	38	61	Numerous, long
	+ Fe 0.05	18	39	69	Numerous, long
	+ 0.11	15	34	51	Numerous, long
	+ 0.23	13	27	42	Few, medium
	+ 0.35	16	35	54	Few, medium

The number and length of side roots are as important criteria with this type of plant as the length of the radical. It appears that small quantities of Fe accelerate growth with respect to distilled water but to no great extent when used alone. It is significant to note that the presence of 0.35 p.p.m. Fe is lethal to the radicals in three days although the tops continue apparently normal. Although side roots appear in solutions containing Fe, their growth is greatly inhibited.

There can be no question of the antagonistic effect of Ca for Fe in the concentrations used; there is not an exception to the contrary. That the toxic action of Fe is not completely antagonized is just as apparent.

It is seen that 0.05 p.p.m. Fe accelerates the growth of beans in the presence of 5.0 p.p.m. Ca. whereas increasing the concentration of Ca with the same amount of Fe produces a growth of radical and side roots as good as that in Ca alone. Within the time limit of the experiment, the toxic effect of 0.11

TABLE 2

Growth of radicals of Lupinus albus in various solutions of Fe and Ca at an average temperature of 17°C.

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	GROWTH DURING 96 HOURS
		mm.	*mm.*	*mm.*	*mm.*
Water		14	21	25	28
	p.p.m.				
Fe	0.11	19	26	29	31
	0.23	17	20	27	28
	0.50	18	23	28	29
	0.70	17	20	24	24
	p.p.m.				
Ca	5.0	26	48	67	79
	+ Fe 0.11	30	49	59	66
	+ 0.23	29	42	54	58
	+ 0.50	25	40	52	56
	+ 0.70	25	48	62	69
	p.p.m.				
Ca	10.0	28	48	62	70
	+ Fe 0.11	26	38	52	65
	+ 0.23	22	40	49	51
	+ 0.50	16	35	52	63
	+ 0.70	24	50	63	68
	p.p.m.				
Ca	33.0	28	45	58	68
	+ Fe 0.11	28	47	62	82
	+ 0.23	27	43	52	61
	+ 0.50	23	40	52	64
	+ 0.70	29	56	76	82

p.p.m. Fe is entirely overcome by 10.0 p.p.m. Ca. On increasing the Fe concentration, slight ill effects are observed no matter how much Ca is added.

Growth acceleration occurs in 10 p.p.m. Ca with an Fe concentration of 0.23 and 0.35 p.p.m. but the number of side roots is less than in Ca alone. When 33.0 p.p.m. Ca is used with Fe, there is a slight decrease in growth of the radical with increased Fe concentration and also a noticeable decrease in the number of side roots although in no case were they observed to be blunted and abortive.

TABLE 3

Growth of radical of Lupinus albus at an average temperature of 23°C. in various solutions of Fe and Ca

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	GROWTH DURING 96 HOURS	GROWTH DURING 120 HOURS	SIDE ROOTS
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	
Water		18.5	24	26	27	27	
	p.p.m.						
Fe	0.2	18.5	28	31	32	32	Normal
	1.0	15	21	25	25	25	Blunt
	2.0	Dead	.....	.....	.....	.....	None
	p.p.m.						
Ca	5.0	22	44	52	55	53	
	+ Fe 0.2	24	42	50	55	56	Normal
	+ 1.0	22	40	52	58	60	Abortive, short
	+ 2.0	11	19.5	22	22.5	23	Blunt

TABLE 4

Growth of radicals of Lupinus albus at an average temperature of 29°C. in various solutions of Ca and Fe

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	SIDE ROOTS AND RADICAL
		mm.	*mm.*	*mm.*	
Water		16	20	23	Loose turgidity (3 days)
	p.p.m.				
Fe	0.2	10.5	14	14	Loose turgidity, markedly (3 days)
	0.5	11.5	15	15	Loose turgidity (2 days), side roots blunt
	1.0	8	10	10	Loose turgidity (2 days), side roots blunt
	p.p.m.				
Ca	5.0	16	25.5	29	Side roots well developed
	+ Fe 0.2	17	27	29	Side roots normal
	+ 0.5	20	33	36.5	Side roots blunt
	+ 1.0	16	24	26	Side roots few and blunt
	p.p.m.				
Ca	10.0	17	30	35	Side roots well developed
	+ Fe 0.2	17	33	39	Side roots normal
	+ 0.5	20.5	33	39	Side roots blunt
	+ 1.0	22	30	32	Side roots few and blunt

TABLE 5

Growth of radicals of Lupinus albus when grown in various solutions of Al, Fe and Ca at an average temperature of 21°C.

SOLUTION		GROWTH DURING 1ST DAY	GROWTH DURING 2ND DAY	GROWTH DURING 3RD DAY	GROWTH DURING 4TH DAY	GROWTH DURING 5TH DAY	GROWTH DURING 6TH DAY	GROWTH DURING 7TH DAY	GROWTH DURING 8TH DAY	
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	
Water		16	23	24	27	27.5	28	28	28	Loss of turgidity in 7 days
	p.p.m.									
Fe	0.5	17	20.5	24	26	27.5	27.0	27.0	27.0	Loss of turgidity in 8 days
	1.0	12.3	18	19.7	21	21	21	20	20	Dead in 7 days
	2.0	Dead							8	
	p.p.m.									
Ca	5.0	24	41	55	62	69	73	76	74	
	+ Fe 0.5	21	39	49	58	62	62	63	63	
	+ 1.0	19	35	43	49	53	56	57	57	
	+ 2.0	12	19	23	26	28	29.5	29.5	29.5	Loss of turgidity in 8 days
	p.p.m.									
Ca	10.0	19	37	50	63	71	73	74	74	
	+ Fe 0.5	20	44	57	67	71	73.5	73	73	
	+ 1.0	20	37	43	48	51	52	52.5	53	
	+ 2.0	13	23	29	32.5	35	37	39	39.5	
	p.p.m.									
Ca	20.0	19	41	56	67	73	77.5	78	78	
	+ Fe 2.0	14	26	32	39	44	47	42	42	
	Al 0.5	15	25	33	41	44	47	47	47	Radical distorted
	1.0	15	29	37.5	41	42	43	43	42	Radical distorted
	2.0	7	7.5	8	8	8	8	8	8	Turgid, dead (?) in 3 days
	p.p.m.									
Ca	5.0	24	41	55	62	69	73	76	74	
	+ Al 0.5	18	32	45	57	66	76	81	82	
	+ 1.0	13.5	29.5	40.5	55	66	75	78	79	Radical distorted
	+ 2.0	11	19.5	27	31	31	31	31	30	Radical distorted
	p.p.m.									
Ca	10.0	19	37	50	63	71	73	74	74	
	+ Al 0.5	16	35	49	62	74	79	83	84	
	+ 1.0	18.5	34	44	52	54	55	54	54	Slight distortion
	+ 2.0	10	19	24	27.5	28	28	28	28	Radical distorted
	p.p.m.									
Ca	20.0	19	41	56	67	73	77.5	78	78	
	+ Al 2.0	13.5	28	40	43	43	43	44	44	Radical distorted

There are at hand five separate experiments on lupines performed from February 1–28, 1928 with Ca and Fe. The concentrations ranged from 0.05 to 2.31 p.p.m. Fe. Some results are given in table 2. In Fe concentrations of 0.05 to 0.5 p.p.m., in single salt solution, there is a rather decided acceleration in the growth rate of the radical for from one to two days depending on the Fe content of the solutions. After the first day, growth closely parallels that in distilled water and the ill effects of such Fe concentrations are no greater than those produced in distilled water. A concentration of approximately 1.15 p.p.m. Fe in single salt solution is required to kill lupines in three days. It is recalled that 0.35 p.p.m. Fe was lethal to Phaseolus in the same time limit. This difference in resistance to Fe parallels the contrasting response of these two plants to distilled water and Ca as before shown.

Table 2 shows again the beneficial action of Ca, but the response with lupines is not so marked as that with beans. (Or perhaps it is better to say that lupines are more resistant to the harmful action of distilled water than are beans.) The antagonism of Ca for Fe is again apparent. Fe used with Ca shows a growth acceleration, as compared to Ca alone, in two places, as was the case with Phaseolus. Concentrations of 0.11 and 0.23 p.p.m. Fe with 5 p.p.m. Ca cause an acceleration of growth as compared to 5 p.p.m. Ca alone for at least 24 hours, the rate being approximate to Ca thereafter. As little as 5.0 p.p.m. Ca seems completely to overcome tne toxicity of 0.5 and 0.7 p.p.m. Fe within the time limit of the experiments. The comparable Fe concentration with Phaseolus is approximately 0.35 p.p.m.

With 33.0 p.p.m. Ca + 0.7 p.p.m. Fe there seems to be a tendency for growth acceleration. Other combinations produce apparently normal radicals.

The data recorded in tables 3 and 5 are typical of the results obtained with lupines during June, 1928. The variation is only in degree. Growth acceleration occurs in 0.2 p.p.m. Fe for three days as compared with distilled water. But increase in growth with 0.5 p.p.m. Fe is noticeable for only 24 hours. Table 2 shows a comparable effect with 0.7 p.p.m. Fe. One p.p.m. Fe is lethal in 7 days and 2.0 p.p.m. Fe in 1 day. The beneficial effects of Ca again appear.

The experiments recorded in tables 3 and 4 were done within two weeks of each other and the only variable is temperature; materials, seeds, and method were exactly the same. At the higher temperature, the toxicity of Fe is more pronounced. A concentration of 0.2 p.p.m. is decidedly toxic, and loss of turgidity results in three days. Growth is distinctly less than that produced in distilled water at this concentration. In an Fe concentration of 1.0 p.p.m., a marked toxic action is observed. A comparison of the figures in tables 3 and 4, indicates that at 29°C., 0.2 p.p.m. Fe is more toxic than 1.0 p.p.m. Fe at 23°C. The influence of temperature is decided.

It is seen also that the response to Ca at the higher temperature contrasts with the reaction at lower temperatures. Lupines profit by increased additions of Ca in single salt solution at 29°C., in which respect they react as Phaseolus at 17–23°C.

At a temperature of 29°C., it is almost impossible to obtain uniform, dependable, and reproducible results with Phaseolus. Generally considered to be hardy plants, they are susceptible to a number of diseases which are particularly active at the higher temperatures. The reaction of Phaseolus at 29°C. would be interesting to know, but it is hardly possible to ascertain by this technique. The results obtained are, therefore, not presented.

Table 5 shows the results of an experiment on lupines as the test plant with the temperature at 21°C. The contrasted effects of Al and Fe are to be noted. Loss of turgidity is a rather convincing criteria of death. Certain concentrations of Fe cause the radical tip to become flabby within several days,

TABLE 6

Effect of various concentrations of Al and Ca on the growth of lupines at a temperature of 29°C.

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	SIDE ROOTS AND RADICAL
Water		16	20	23	Loss of turgidity in 3 days
	p.p.m.				
Al	0.2	15	22	24	Side roots few and blunt
	0.5	15	24	26	Tips very scaly
	1.0	12	17.5	18	Cortex contracted 5 mm. from tip
	2.0	6.5	6.5	6.5	Apparently dead
	p.p.m.				
Ca	5.0	16	25	29	
	+ Al 0.2	16.5	33	40.5	Nearly normal
	+ 0.5	16	28	31	Distortion, side roots few and blunt
	+ 1.0	17	26	28	Distortion, side roots few and blunt
	+ 2.0	9	13	13	In 24 hours tip very brittle and scaly
	p.p.m.				
Ca	10.0	17	30	35	
	+ Al 0.2	18	34	41	Normal
	+ 0.5	15	24	29	Approach normality
	+ 1.0	14	18	19	Distorted, side roots blunt
	+ 2.0	12	18	20	In 24 hours tips scaly but not brittle

as already pointed out. But in no case with Al was the radical tip observed to be in the least altered in this regard. The ultimate toxic action differs greatly from that with Fe. Grown in Al solutions of 0.5 p.p.m. or more, the radicals bend in all directions and are greatly distorted, the distortion apparently increasing with increase in Al concentration. This phenomenon was not observed in the case of Fe. In single salt solutions of Al, the epidermis of the radicals was observed to become scaly and very brittle. The brittle character apparently continued into the cortex causing the radicals to break wherever they bent unless great care was taken. Fe was never observed to have this effect. The criteria of death with Al is not apparent to the writer.

The following peculiarities in growth are noted: Used in single salt solution, Al causes a great growth stimulation to the radical in comparison with distilled water. This contrasts sharply with Fe at the same concentrations (0.5 and 1.0 p.p.m.). With Ca, it is seen that 0.5 and 1.0 p.p.m. Al allow nearly normal growth of radicals after, perhaps, the first day. It would almost seem that the presence of Al were beneficial to lupines.

But with a concentration of 2.0 p.p.m. Al, a rather direct comparison of toxicity with Fe is obtained. Used alone or with 5.0, 10.0, or 20.0 p.p.m. Ca, the rate of growth compares with that in the various solutions of Fe. Used in sufficient concentration, Al is approximately as toxic as Fe.

Table 6 shows the effect of various concentrations of Al and Ca on the growth of lupines at a temperature of 29°C.

The growth depression with increase in temperature is again observed. Compared with distilled water, 1.0 p.p.m. Al is significantly toxic. At 21°C. an excellent growth was obtained at the same concentration. A parallel effect has been shown for Fe. The antagonistic action of Ca appears again although not so efficiently as at the lower temperature.

Comparing the results of table 6 with those of table 4 (temperature in both cases 29°C.), it is seen that Al depresses growth less than does Fe at the same concentrations and even when the initial growth depression with Al is of the same order as with Fe, elongation continues in the former solutions while ceasing in the latter. The results reported in table 5 are checked at the higher temperatures.

DISCUSSION

The response of lupines and Phaseolus to distilled water and Ca has been discussed in a previous paper (16). This paper concerns itself with the varied behavior of the plants to Fe and Al; to the antagonism of Ca for these elements; and the possible effect of temperature on toxic action.

1. At temperatures of from 17–21°C., in the same time interval, 0.35 p.p.m. Fe is toxic and lethal to beans while a concentration of 1.15 p.p.m. is lethal to lupines. It requires about three times the Fe concentration to produce the same result with lupines as with beans. This relative sensitivity of the two plants is seen throughout the work and parallels the sensitivity to distilled water.

Calculated to molar concentrations, there is much evidence (8, 10, 16) that the toxicity of heavy metals is greater than the toxicity of free acid. At a pH of 4.5, Phaseolus just about survives, that is to say at a molar HCl concentration of 0.00003. The same plant just survives with a molar Fe concentration of 0.000004. It would seem that the Fe ion is approximately seven times as efficient as the H-ion in its killing action.

The record for lupines will not permit such close comparison. They are known to survive for one day at a pH of 3.8 and for at least four days with a pH of 4.1. The limit of toleration is approximately 0.0001 *M* HCl. With Fe,

survival is at a molar concentration of 0.000018 (as chloride). With this plant, it again appears that the Fe ion is much more toxic than the H ion. Were the calculations in terms of normalities, the difference in toxicity would be multiplied by three, but the weight of evidence indicates that the effect is not due to valence in the case of heavy metals.

The writer offers no explanation for the different physiological toxicity of Fe and Al. That Al is lethal to plants cannot be denied, but why the root tips do not loose their turgidity is not apparent. In weak concentrations Al seems less toxic than Fe; stimulation of growth would suggest this. Work has been done to explain this action but no definite evidence has been obtained. The opinion is suggested that Fe is relatively immobile while Al is mobile. Kratzman (11) has shown the presence of Al in 130 plants species and finds no correlation of the relative presence or absence with plant families. His method indicates that Al is stored in plant cells in much the same manner as calcium oxalate crystals are found, and he suggests that "some plants may justly be called aluminum plants." Hoffer and Carr (9) have demonstrated the mobility of Al in corn. McHargue (14) and Hoffer and Carr (9) have demonstrated the presence of Fe in seeds; and its presence in chloroplasts can be demonstrated with little trouble at any time. This question seems to the writer to be indissolubly linked with permeability, not only of peripheral cells but of contiguous cells.

2. It is doubtful how far these data can be used to explain why one soil with low pH is toxic whereas another is not. That Ca is a factor is reasonable to suppose, but there are many others. However some evidence is at hand bearing on this point.

Six-inch pots were filled in duplicate with six soils [for analysis of four soils see previous paper (16)]. Five of these soils were known to lack plant-food and to be low in Ca; the fifth was regularly limed every year. Each pot was planted with 12 bean seedlings. One series was watered with distilled water, the second and third with 3.3 p.p.m. Fe. But to the third series had been added 5 gm. of $CaCO_3$ per pot. Approximately 14 liters of solution were used to each pot added at 15 intervals. Table 7 shows the tendency.

In all cases the presence of free Fe in the soil solution produced a stunted crop, and, with one exception, the addition of Ca to the soils almost entirely overcame the toxicity of the Fe. The use as a check on this point of a soil which had been limed for several years sustains the opinion that lime in the soil solution is an important ecologic factor even when the pH is abnormally low, and further, that lime in the soil solution may completely antagonize the toxic action of traces of Fe. The antagonism of Ca for Al has been shown.

From the data presented, it is doubtful if Phaseolus can survive an Fe or Al concentration exceeding 0.35 p.p.m. no matter how much Ca be present. With lupines the limit is placed at about 1 p.p.m. Fe or Al with the presence of a minimum concentration of Ca.

That Al is present in the soil at about this concentration, although not

demonstrated, is to be expected from the work of Magistad (15). Fe parallels the solubility of Al to a great extent. With the turning point of an Fe and Al solubility curve at pH 5.0, the two metals are brought into the soil solution in increasing concentration depending on the phosphate content of the soil. These metals are about five times as efficient in their toxic action as the H ion but their toxic action will depend on the Ca concentration of the soil solution.

TABLE 7

Weight of tops of Phaseolus sp. grown in pots with six different soils

One soil series is untreated; the second and third series show the effect of Fe and the antagonism of Ca.

SOIL	DISTILLED WATER ONLY			3.3 P.P.M. Fe (NO $CaCO_3$)			3.3 P.P.M. Fe, 5 GM. $CaCO_3$ PER POT		
	Number plants per pot	Average weight tops 1 plant	Nodules	Number plants per pot	Average weight tops 1 plant	Nodules	Number plants per pot	Average weight pots 1 plant	Nodules
		gm.			*gm.*			*gm.*	
1	9	3.5		10	3.2		11	3.5	
1	11	4.1		10	2.8		11	3.7	
Average......		3.8	Few		3.0	None		3.6	Many
2	11	4.6		11	3.9		11	3.9	
2	11	4.1		11	3.6		10	3.4	
Average......		4.35	Many, small		3.75	Few, small		3.65	Very many, large
3	11	3.5		10	2.2		10	3.2	
3	10	4.0		11	2.3		10	3.7	
Average......		3.75	None		2.25	None		3.45	Many, large
4	11	3.4		10	2.8		10	3.9	
4	11	3.5		11	3.3		11	4.0	
Average......		3.45	None		3.05	None		3.95	Many, large
Limed soil	11	4.2	Many	10	4.2	Many, small	11	4.2	Very many, large
5	8	3.7	None	10	2.8	None	9	3.9	Many, small

From Magistad's curve and from the data here presented, it is quite possible that a soil with a pH of 4.3 will show no evidence of toxicity should the phosphate and Ca content be high. Blair and Prince (3) and Burgess (5) have indicated that with lettuce, onions, and soybeans, the toxicity of soils is not due to pH but most probably to Al.

3. Leitch (13), Lehenbauer (12), Balls (1), and others have shown that the optimum temperature for the growth of plants is 29°C. From 2–29°C.,

growth and temperature can be expressed in a uniform curve; at higher temperatures the growth curve is very erratic, and no single law governs the tendency.

Behring (2) showed that the action of disinfectants is increased by a rise in temperature but the writer knows of no other work bearing on the increased toxicity of metals with rise in temperature.

The data presented show the increased toxicity of water at 29°C., the greater toxic action of Al and Fe at this temperature, and the marked beneficial effects of increased concentrations of Ca. The resistant lupine acts at 29°C. much the same as the relatively sensitive bean at 17–21°C. These data check the demonstrations of Hansteen-Cranner (6) but a discussion is reserved until more evidence is obtained.

It is the opinion of the writer, from the evidence presented, that the value of pH determinations of the soil is only in the prediction of the elements one may expect to find in the soil solution; that plants do not show a preference for a specific pH in the concentrations in which the H ion is usually found in the soil (16) but that plants may more correctly be called "calciphils" and "calciphobes" or, from Kratzman's investigations, "aluminaphils" and "aluminaphobes."

SUMMARY

With the condition and growth of the radicals of *Lupinus albus* and *Phaseolus vulgaris nanus* as criteria, the relative toxicity of Fe and Al to these plants and to each other was studied.

Some data are presented showing the increased toxicity of Al and Fe at 29°C.

L. albus is about three times as resistant to Al and Fe as is *Phaseolus*.

The Fe ion is about five to seven times as toxic as the H ion.

The different physiological toxicities of the Al and Fe ions are designated.

The antagonism of Ca for Al and Fe is demonstrated and from these data the limits of Al and Fe concentration that may exist in the soil solution are approximated for *L. albus* and for Phaseolus. Pot experiments are used to check water culture conclusions.

Experiments and citations are discussed in support of the opinion that cH[1] of itself is of little importance as an ecologic factor.

REFERENCES

(1) Balls, L. W. 1908 Temperature and Growth. *Ann. Bot. (London)* 22: 557–591.

(2) Behring, 1890 Ueber Desinfection, Desinfectionsmittel, and Desinfectionsmethoden. *Ztschr. Hyg. u.* Infektions krank. 9: 395–478.

(3) Blair, A. W., and Prince, A. L. 1923 Studies on the toxic properties of soils. *Soil Sci.* 15: 109–129.

[1] cH is the symbol for H-ion concentration. $pH = \log \frac{1}{cH}$.

(4) BLAIR, A. W., AND PRINCE, A. L. 1927 The relation of soil reaction to active aluminum. *Soil Sci.* 24: 205–213.
(5) BURGESS, P. S. 1923 A method for the determination of active aluminum in acid soils. *Soil Sci.* 15: 131–135.
(6) HANSTEEN-CRANNER, B. 1919 Beitrage Zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. *Ber. Deut. Bot. Gesell.* 37: 380–391.
(7) HARTWELL, B. L. AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *Soil Sci.* 6: 259–279.
(8) HEALD, F. D. 1896 On the toxic effect of dilute solutions of acids and bases upon plants. *Bot. Gaz.* 22: 125–153.
(9) HOFFER, G. N., AND CARR, R. H. 1923 Accumulation of aluminum and iron compounds in corn plants and its probable relation to root rots. *Jour. Agr. Res.* 23: 801–824.
(10) KAHLENBERG, L., AND TRUE, R. H. 1896 On the toxic action of dissolved salts and their electrolytic dissociation. *Bot. Gaz.* 22: 81–124.
(11) KRATZMAN, E. 1913 Der mikrochemische Nachweis und die Verlveitung des Aluminiums in Pflanzenreich. *Sitsber. K. Akad. Wiss. (Vienna), Math. Naturw. Kl.* 122: 311–336.
(12) LEHENBAUER, P. A. 1914 Growth of maize seedlings in relation to temperature. Abs. in *Physiol. Res.* 1: 247–288.
(13) LEITCH, T. 1916 Some experiments on the influence of temperature on the rate of growth of Pisum sativum. *Ann. Bot. (London)* 30: 25–46.
(14) McHARGUE, J. S. 1923 Iron and manganese content of certain species of seeds. *Jour. Agr. Res.* 23: 395–399.
(15) MAGISTAD, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181–226.
(16) SKEEN, J. R. 1929 Some reactions of seedlings to weak concentrations of HCl and Ca. *Soil Sci.* 26: 471–478.
(17) WHERRY, E. T. 1927 The soil reaction preferences of certain plant orders. *Wash. Acad. Sci. Jour.* 17: 148–149.

BOOK REVIEW

Lehrbuch der Agrikulturchemie (*Textbook of Agricultural Chemistry*). By E. HASELHOFF and E. BLANCK. Gebrüder Borntraeger, Berlin. Teil I. *Pflanzenernährungslehre* (*Plant Nutrition*). By E. BLANCK. 1927. Pp. 207, price, 10.50 M. Teil II. *Düngemittellehre* (*Fertilizers*). By E. HASELHOFF. 1928. Pp. 216, price, 12.0 M. Teil III. *Bodenlehre* (*Soils*). By E. BLANCK. 1928. Pp. 208, price, 11.40 M.

The purpose of these books, which are designed primarily for the agricultural student and the practical agriculturist, is to give a summary of the present status of investigation in the field of agricultural chemistry. Without going into a detailed review of the various individual investigations, the authors have presented a complete survey of the science of agricultural chemistry and its influence upon practical agriculture. No attempt has been made to review the literature completely or thoroughly; the authors have limited themselves to a discussion of the fundamental principles underlying the subject.

The first part, prepared by Prof. Blanck of the University of Göttingen, treats of the principles of plant nutrition and plant metabolism, including sections on the chemical composition of the organic and inorganic plant constituents and on the formation and transformation of organic matter in the plants. The second part, prepared by Prof. Haselhoff, deals with principles of fertilization and with natural and artificial fertilizers. The third part, prepared by Prof. Blanck, treats of soil science in its relation to geology, processes of weathering, the rôle of minerals and rocks in soil formation, influence of climate upon soil formation, chemical composition of soil, biological condition of soil, etc. The fourth part of this book dealing with animal feeding, by E. Haselhoff, is still to be published.

Each part is complete in itself and has detailed author and subject indexes. The treatment of the subject is authoritative throughout and the book should find its place in every agricultural library.

S. A. WAKSMAN.

SOIL SCIENCE

Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

EDITORIAL BOARD

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to Dr. Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

Soil Science is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Volumes begin with the January and July issues, respectively. The subscription price is $10.00 for Volumes XXV (Nos. 1–6 inclusive) and XXVI (Nos. 1–6 inclusive) January, 1928, to December, 1928, inclusive, United States, Mexico, Cuba. An additional charge per volume of 50 cents for subscriptions in other countries. *Back volumes* are supplied on orders for Vols. I to XIV inclusive, XIX to XXIV incl.—$100.00 per set.

New subscriptions are accepted to begin with the current issue, unless the subscriber gives instructions to begin the subscription with some other issue. If the subscriber desire, back issues of the current volume may be obtained when in stock. The publishers do not guarantee to supply back issues, however.

Renewal subscriptions. To avoid a break in your series, subscriptions should be renewed promptly on expiration. Otherwise it may prove impossible for the publishers to furnish the back issues missed, as only sufficient copies are printed of each issue to supply orders in hand.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; or G. Jervis Manton, 430 Lit. Collins St., Melbourne (and at Sydney and Brisbane).

For the British Empire, except Australia and Canada: Bailliere, Tindall & Cox; 8 Henrietta Street, Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann, Krausenstrasse 38, Berlin N. W., Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

Publishers of: *Medicine, Journal of Urology, Bulletin of the Johns Hopkins Hospital, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Physical Therapeutics, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thompson Research Laboratory, Journal of Biological Chemistry, Chemical Reviews, Journal of Dairy Science, Soil Science, Social Forces, Personnel Journal, Journal of Comparative Psychology, Comparative Psychology Monographs, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, American Speech, Childhood Education.*

THE INFLUENCE OF MANGANIFEROUS SOILS ON THE ACCURACY OF THE QUINHYDRONE ELECTRODE

W. T. McGEORGE

Hawaiian Sugar Planters' Association Experiment Station

Received for publication August 20, 1928

The increasingly important position of soil reaction in soil fertility studies has stimulated much development in accurate methods of estimating slight variations in hydrogen- and hydroxyl-ion concentration in soils. The investigations of Sharp and Hoagland (7) on the application of the hydrogen electrode stand out prominently in this field of endeavor. But, as usually follows the extensive application of new analytical methods, limitations are often met in practice. In measuring the pH of neutral or slightly alkaline soils, the CO_2 equilibrium enters into the reaction and the observed voltages are often misleading. In some cases, hydrogen must be passed through the soil for long periods which too, is an objection. Also the electrode may show error in the presence of certain unsaturated substances or small amounts of active oxidizing agents, such as nitrate ions. Some have registered objection to the platinum black coating on the electrodes which is rapidly poisoned by certain materials.

In 1920, Billman showed that it was possible to form, by means of the organic compound quinhydrone, an electrode which could be used for making hydrogen-ion determinations and with which very constant voltages could be maintained. Out of this, the so-called quinhydrone electrode method for determining soil reaction has grown. The method was shown to have several advantages over the hydrogen electrode, notable among which were its simplicity, rapidity with which determinations could be made, and the fact that clean platinum electrodes were used. It is now being extensively applied in determining the reaction of soils.

The theory of the quinhydrone electrode has been ably stated and reviewed in a number of recent articles so will not be repeated here (1, 2, 3, 4). Suffice it to say, that the validity of the method is based largely upon the dissociation products of quinhydrone in aqueous solutions; namely, quinone, $C_6H_4O_2$, and hydroquinone, $C_6H_4O_2H_2$. The equilibrium of these products of dissociation is an essential feature in the accuracy of the method. It is the purpose of this article to present what appears to be a limitation in the application of the quinhydrone electrode in determining the reaction of soils.

EXPERIMENTAL

The quinhydrone electrode was prepared as described by Billman (2, 3). The special KCl–HCl half cell (0.01 *N* HCl : 0.09*N* KCl) was used. This was

connected by capillarity with a saturated KCl solution and this in turn by a KCl-agar bridge to the soil-water suspension to which a given weight of quinhydrone was added. Clean platinum foil was used for the electrodes. The difference in potential was measured with an L. N. students' type potentiometer and a sensitive galvanometer. Correction was made for temperature as in the following equation:

$$\text{pH} = \text{pH of KCl} - \text{HCl half cell} + \frac{\text{E.M.F.}}{0.000198\ T}$$

The same potentiometer and galvanometer were used in the comparative determinations with the hydrogen electrode.

The soils selected represented a wide variation in Hawaiian soil types and a reaction range of pH 4.6 to 8.0. A brief description of the soils follows:

1. A reddish yellow clay loam, acid soil, low fertility.
2. A black silty clay loam.
3. A yellowish brown silty clay loam.
4. A yellow silty clay loam.
5. A brown silty clay loam.
6. A chocolate brown silt loam, highly manganiferous type.
7. A heavy black clay, high magnesium soil, adobe type.
8. A black silt loam, highly organic.
9. A yellow silty clay loam.
10. A brown silty clay loam.
11. A yellow silty clay loam.
12. A red silty clay loam.
13. A silt loam, highly organic.
14. A reddish brown silty clay loam.
15. A red clay loam.
16. A brown silty clay loam.
17. A yellowish brown silty clay loam
18. A highly saline clay soil.
19. A red clay loam.
20. A red clay loam.
21. A red clay loam.
22. A red clay loam.

For the pH determinations, all soils were air-dry. Only boiled distilled water was used for the soil-water suspensions. The ratios of soil to water were 1:1, 1:2, and 1:5. On account of the high water-absorbing power of many Hawaiian soils, it is difficult to get a reading with the hydrogen electrode on a 1:1 ratio. Even where the mixture is of sufficient fluidity, the potential is very slow in reaching equilibrium, which may account for some of the discrepancies in this column of the table.

For the determinations with the hydrogen electrode the soil-water suspension was prepared one hour before the pH readings were taken. The soil-water suspensions for the quinhydrone electrode were also prepared one hour in advance, but the reagent was added immediately before the reading was taken.

During some preliminary tests, it was found that in most cases, 0.025 gm. quinhydrone was sufficient to give a constant reading whereas in others, larger amounts were required and there was a notable "drift." The procedure employed was to add 0.025 gm. of quinhydrone and take the reading at once. Another 0.025 gm. was then added and a second reading taken. This was continued until there was no further drift in the potential. The data are given in table 1 and shown graphically in figure 1.

TABLE 1

The pH of soils by hydrogen and quinhydrone electrodes at 1:1, 1:3, and 1:5 soil-water ratios compared with percentages of manganese in soils

SOIL NUMBER	HYDROGEN ELECTRODE			QUINHYDRONE ELECTRODE			PER CENT Mn_3O_4
	1:1	1:3	1:5	1:1	1:3	1:5	
1	4.63	4.65	4.75	4.38	4.81	4.86	0.05
2	4.83	4.76	4.95	4.73	5.05	5.03	0.02
3	4.88	4.92	5.14	4.80	5.15	5.24	0.04
4	4.93	5.14	5.22	4.73	5.22	5.37	0.07
5	5.19	5.48	5.61	5.41	5.54	5.58	0.04
6	5.61	5.82	6.05	7.28	7.46	7.72	4.53
7	4.97	5.76	6.23	6.10	6.26	6.71	0.06
8	5.31	5.78	5.68	5.66	5.87	5.83	0.09
9	5.42	5.76	5.91	5.68	5.79	5.83	0.07
10	5.47	5.76	5.98	5.75	5.92	5.92	0.13
11	5.81	6.19	6.37	6.08	6.26	6.30	0.13
12	6.12	6.39	6.44	7.20	7.63	7.70	0.34
13	6.32	6.25	6.74	6.60	6.66	6.68	0.03
14	6.66	6.79	6.88	6.72	6.85	6.88	0.07
15	6.66	6.86	7.15	7.88	8.14	8.06	0.20
16	6.96	6.90	7.05	7.16	7.31	7.38	0.08
17	6.57	6.88	7.10	6.76	6.96	6.97	0.13
18	7.50	7.71	8.01	7.79	8.00	8.14	0.03
19	7.81	7.50	7.67	8.02	8.27	8.36	0.13
20	7.38	7.42	7.50	8.36	8.48	8.54	0.33
21	7.34	7.52	7.45	8.11	8.44	8.36	0.16
22	7.25	7.34	7.08	8.11	8.31	8.36	....

The data in table 1 and in figure 1 show several soils in which there is a decided lack of agreement and among these is the highly manganiferous type. Manganese was then determined in all the soils by extraction with HCl, sp. gr. 1.115, and these data have been added to the table. By reference to the description of the soils, it will also be noted that in all cases where lack of agreement was found, the soil was a red clay type. Such types we consider to have been formed in a more arid environment, as distinguished from the humid, and therefore contain iron and manganese as the higher oxides. Manganese is present in such types in the form of small pellets of MnO_2 or as a coating of MnO_2 upon the surface of the soil particles. The evidence is therefore quite convincing

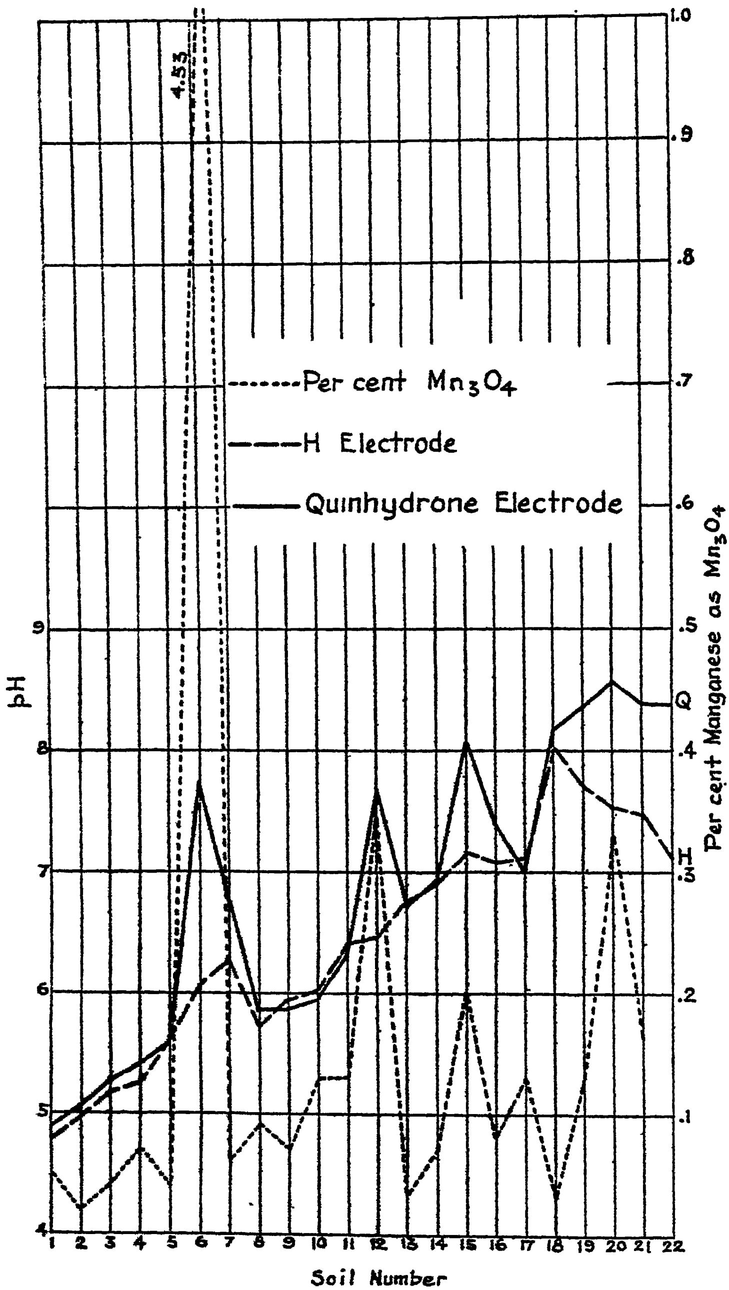


FIG. 1. RELATION OF MANGANESE CONTENT OF SOIL TO pH AS DETERMINED BY QUINHYDRONE AND HYDROGEN ELECTRODES

that MnO_2 materially disturbs the equilibrium of the dissociation products of quinhydrone.

In view of this soil-water suspensions were prepared to which were added manganese dioxide, ferric oxide, and ferric hydroxide. The two latter had no effect upon the potentiometer reading. On the other hand, manganese dioxide produced a "drift" similar to that shown by the "truant" soils. These data are given in table 2.

The conclusion appears inevitable that the quinhydrone electrode is in error as applied to soils containing even small amounts of manganese dioxide. As a further check upon this, the two electrometric methods were compared with the colorimetric method. Of the 22 soils given in table 1, only 5 settled sufficiently to give a supernatant water extract which could be used for a colorimetric comparison. These soils were numbered 6, 8, 12, 16, and 18. In all five soils, the colorimetric and hydrogen electrode methods checked closely,

TABLE 2

Potentiometer readings in E.M.F.

MnO_2 ADDED	0.025 GM. QUINHYDRONE	0.050 GM. QUINHYDRONE	0.075 GM. QUINHYDRONE	
gm.				
None	0.130	0.130	0.130	Soil I
0.2	0.180	0.290	0.295	
0.5	0.230	0.295	0.300	
None	0.245	0.245	0.245	Soil II
0.2	0.295	0.345	0.345	
0.5	0.320	0.360	0.355	

whereas the quinhydrone electrode gave results much higher in 6 and 12, the manganiferous soils.

DISCUSSION

In the original work by Billman, 75 soils were used and comparisons between the hydrogen and quinhydrone electrodes made. In all except 7, close agreement was found, and in these 7 soils the disagreement was later found to be due to analytical errors. No mention is made as to any other factor arising in these discrepancies. Baver (1) has obtained satisfactory results, as has also Clark and Collins (4), with the quinhydrone electrode.

La Mer and Parsons (5), and La Mer and Rideal (6), have made rather intensive studies of the factors affecting the accuracy of the quinhydrone electrode which are of interest in connection with the above property of manganiferous soils. They found that the addition of an alkali to a solution of quinhydrone causes the latter to act as an auto-oxidizable substance according to the following equation:

$$C_6H_4(OH)_2 + O_2 \rightarrow C_6H_4O_2 + H_2O_2$$

In other words, that hydroquinone has the property of absorbing oxygen in alkaline solution. But, when the solution is acid, molecular oxygen is unable to effect any detectable oxidizing action upon hydroquinone although the latter is rapidly oxidized by certain *inorganic agents* possessing less powerful oxidizing potentials than oxygen, such as ferric ions. As previously stated, the validity of the quinhydrone method depends primarily upon the ratiò of quinone to hydroquinone equal to unity. La Mer and Parsons name three factors which may cause this equilibrium to be disturbed.

1. The ionization of hydroquinone as a weak acid . . . .

2. Through hydroquinone or quinone being converted into the other by the action of some other active oxidizing or reducing component present in the system or by their unequal conversion into other bodies. The presence of such rapid oxidizing agents as ferric, permanganate, and dichromate ions or such rapid reducing agents as iodide, titanous, and chromous ions, to mention only a few, will obviously interfere with the use of the quinhydrone electrode, as all these substances react so rapidly with hydroquinone or quinone that they may be used to determine them in volumetric analysis.

3. Changes due to the presence of other electrolytes . . . .

CONCLUSIONS

The accuracy of the quinhydrone for the determination of soil reaction is greatly affected by the presence of small amounts of manganese dioxide.

It is not the purpose of this article to condemn the use of the quinhydrone electrode for soil analyses. The drift shown by the potentiometer with manganiferous soils is sufficiently rapid and characteristic as to warn the analyst against reporting erroneous results on such types.

REFERENCES

(1) Baver, L. D. 1926 The use of the quinhydrone electrode for measuring the hydrogen-ion concentration of soils. *Soil Sci.* 21: 167–179.

(2) Billman, E. 1924 On the measurement of hydrogen-ion concentration in soil by means of the quinhydrone electrode. *Jour. Agr. Sci.* 14: 221–232.

(3) Billman, E., and Tovborg-Jenson, S. 1927 On the determination of the reaction of soils by means of the quinhydrone electrode. *Trans. 2nd Comm. Internat. Soc. Soil. Sci.* B: 236–274.

(4) Clark, N. A., and Collins, E. R. 1927 The quinhydrone electrode and the soil reaction. *Soil Sci.* 24: 453–462.

(5) La Mer, V. K., and Parsons, T. R. 1923 The application of the quinhydrone electrode to electrometric acid-base titrations in the presence of air, and the factors limiting its use in alkaline solutions. *Jour. Biol. Chem.* 57: 613–631.

(6) La Mer, V. K., and Rideal, E. 1924 The influence of hydrogen-ion concentrations on the auto-oxidation of hydroquinone. A note on the stability of the quinhydrone electrode. *Jour. Amer. Chem. Soc.* 46: 223–231.

(7) Sharp, L. T., and Hoagland, D. R. 1916 Acidity and absorption in soils as measured by the hydrogen electrode. *Jour. Agr. Res.* 7: 123–145.

MINIMUM POTASSIUM LEVEL REQUIRED BY TOMATO PLANTS GROWN IN WATER CULTURES

EARL S. JOHNSTON AND D. R. HOAGLAND

University of Maryland and University of California[1]

Received for publication September 21, 1928

Concentration and volume are two distinct characteristics of a solution that should receive equal consideration in studies on plant nutrition. Too frequently the concentration of a solution is discussed without proper consideration of the volume involved. These two factors are in part analogous to the "intensity" and "capacity" factors of energy. The energy of a falling stream of water depends on the height of fall, the *intensity* factor, and on the quantity of water falling, the *capacity* factor. In a nutrient solution the initial concentration may be considered the intensity factor and the volume or total available supply, the capacity factor. Each is fundamental and should be considered in interpreting the results of plant nutritional experiments. In the soil these two characteristics are likewise important. The intensity factor may be regarded as the concentration of the soil solution at any given time, whereas the capacity factor is determined by the nature of the solid phases and by the volume of soil physiologically available for root dispersion.

An important question to be answered regarding the mineral nutrient requirements of plants and one which is not only scientifically interesting but also directly related to practical agriculture is, "What is the optimum concentration of a given element in a nutrient solution when the volume or total supply is not a limiting factor?" The term "optimum solution" here used is defined (4) as the "least concentration giving a yield equal to any higher concentration," that is, a minimum concentration giving a maximum yield. It is of course understood that such a question must be answered first for a particular plant grown under a given set of conditions, and later for other plants grown under other conditions.

Analyses of published data on the effect of concentration on plant growth frequently show a volume relationship. The total salt supply often becomes the limiting factor. Before it is possible to determine accurately the effect of concentration it seems necessary to eliminate or to minimize the factor of volume or total supply from the problem. The use of large volumes of solution as well as frequent changes is a move in the right direction. Parker and Pierre (12) conducted an experiment to determine the minimum concentration

[1] Acknowledgment is made of a grant from the American Potash and Chemical Company in partial support of this investigation.

of potassium necessary for maximum growth of corn and soybeans. One corn plant was grown in 7500 cc. of nutrient solution which was changed twice a day during the later part of the growing period when absorption was rapid. Under these conditions the average rate of change was approximately 10 cc. per minute per plant.

A few experiments have been reported in which solutions were continuously changed, but in these experiments the rates of flow per plant were too low to to make an accurate study of the minimum concentration. Trelease and Livingston (17) report a rate of 400 cc. per day per plant, which is approximately 0.3 cc. per minute. Shive and Stahl (14) have described and used a much simplified apparatus for the continuous renewal of solutions in a series of cultures. The delivery tubes of their apparatus were calibrated to supply each culture with 1 liter of new solution every 24 hours. If each culture contained a single plant the rate of flow would be approximately 0.7 cc. per minute. This apparatus has the advantage of simple construction and of being well adapted to the simultaneous study of a large number of solutions. Its capacity as described is not great enough for studies of minimum salt requirement, but it could doubtless be employed for such studies by using larger reservoir bottles and delivery tubes of greater bore.

EXPERIMENTATION

The experiments reported in this paper were designed to approximate the minimum potassium concentration required by the tomato plant. The work was carried out in the division of plant nutrition at the University of California with the Santa Clara Canner variety. Seeds were germinated between layers of moist filter paper and when the roots were 2 to 10 mm. long the young plants were transferred to a germination net similar to that previously described (8). After the seedlings had developed good roots and grown to approximately 2 to 3 cm. in length they were transferred to the culture solutions.

A special flowing solution apparatus, already described (9), was devised for these experiments. The plant containers were 5-gallon glazed earthenware jars. A special cover of heavy sheet copper was made to fit each of the 24 jars used in these experiments. Five holes were cut in each cover and then a heavy coating of tin was applied as a precaution against copper poisoning. These holes were large enough to receive the flat cork stoppers commonly used to support plants in ordinary fruit jars. The rate of flow of solution from the plant container was controlled by raising or lowering a siphon outlet which was fitted to each culture jar. The appearance of the apparatus at the beginning of one of the experiments is shown in plate 1, figure 1.

The general culture solutions used in the flowing solution experiments were made up from the following salts: calcium nitrate, magnesium sulfate, magnesium phosphate (secondary), manganese sulfate, and ferric tartrate. Since boron was found by Johnston and Dore (10, 11) to be absolutely necessary for the growth of the tomato plant, it was added as boric acid. To the general

culture solutions potassium was supplied as potassium sulfate. In the first experiment the approximate calculated concentrations of these nutrient ions were:

	SOLUTION A		SOLUTION B	
	p.p.m.	*milliequivalent**	*p.p.m.*	*milliequivalent**
Ca	200	10.0	200	10.0
Mg	67	5.5	67	5.5
K	*3.9*	0.1	*39.1*	1.0
NO_3	620	10.0	620	10.0
SO_4	199	6.3	242	7.8
PO_4	15	0.5	15	0.5
B	0.55	0.15	0.55	0.15
Mn	1	0.0364	1	0.0364
Fe	(Enough to keep plants green)			

*Values based on a liter of solution.

Actual analyses, however, showed these general culture solutions to contain less potassium than that calculated. The initial potassium concentration of the solutions used in the five experiments here reported are expressed as average values of several analyses. The solutions used in the other experiments were like these with the exception of the amounts of potassium sulfate.

The particular potassium concentrations employed were not chosen entirely arbitrarily, but the values were suggested by the magnitude of potassium concentrations found in the displaced solutions of certain soils which were being investigated with reference to the adequacy or inadequacy of their potassium-supplying power. It was essential to limit the experiments to one general type of culture solution, and therefore we are not now considering the possible physiological effects of different concentrations of other ions in solutions containing very low potassium concentrations. It need only be remarked that the solution used was in a general way very similar to many displaced solutions obtained from California soils.

Growth data

In the first of the five experiments with the flowing solution apparatus the 24 culture jars each containing four plants were divided into four groups. In order to study the possible relations between light conditions and potassium concentrations the plants in two groups were grown under a single thickness of cheesecloth within the greenhouse, the others were grown under ordinary greenhouse conditions. One group of plants in the cheesecloth shelter and one group of unshaded plants were supplied with the low potassium solutions (3.7 p.p.m.) whereas the other two groups received the high potassium solution (35.1 p.p.m.). For brevity these four culture groups are designated as *OH*, *OL*, *SH*, and *SL* where *O* represents open or unshaded conditions in the greenhouse; *S*, shaded conditions; *H*, high potassium; and *L*, low potassium. Thus

SH represents the group of plants grown in high potassium solution within the cheesecloth shelter in the greenhouse.

The seedlings of the first experiment were set out in their respective culture jars on August 17, 1926. The approximate rate of solution flow per plant per minute was 1.2 cc. By September 16, differences between plants of the high and low potassium groups were quite marked. Both the *OH* and *SH* plants were taller and had thicker stems. Flower buds were noted on the plants of the *OH* group on September 20 and on those of the *OL* group on September 25. On September 22 and October 2 samples of the solutions from the culture jars were taken for potassium analysis. These data are represented in table 1.

The solutions before they entered the culture jars had a pH value of 7.2 and the average values of the potassium analyses gave 35.1 p.p.m. and 3.7 p.p.m. for high and low cultures respectively. The figures given in table 1, when the growth of the plants is considered (see table 2), clearly indicate that the low potassium concentration used in this experiment with the given rate of flow (1.2 cc. per minute) was a limiting factor. The analyses of the residual solutions on September 22 show that the plants removed enough potassium to reduce the concentration from 35.1 to 13.2 and 10.7 p.p.m. in case of the high potassium groups, *OH* and *SH*, and from 3.7 to 0.2 and 0.3 in the low potassium groups, *OL* and *SL*.

TABLE 1

Potassium analyses of flowing nutrient solutions in which tomato plants were growing

CULTURE	SEPTEMBER 22		OCTOBER 2	
	pH	*K(p.p.m.)*	*pH*	*K(p.p.m.)*
OH	7.2	13.2	7.4	8.5
OL	7.2	0.2	7.2	0.7
SH	7.4	10.7	7.4	11.6
SL	7.4	0.3	7.0	0.4

The plants were harvested on October 5 and 6; these data are given in table 2. The general appearance of the *OH* and *OL* groups prior to harvest can be seen in plate 1, figure 2. The *SH* and *SL* groups are within the cheese cloth shelter. The values for each group represent the average of 24 plants except in the case of the dry weight of tops, where 12 plants were used, the other 12 having been set aside for other purposes. Stem height was considered to be the distance from the upper surface of the cork stopper to the base of the terminal bud. At the time of harvest numerous flower buds were present on the plants of the *OH* group with full flowers appearing on about half the number. A few flower buds, none open, however, appeared on the plants of the *OL* group, but none was observed on any plants of the shaded groups.

Dry weight data (table 2) are perhaps the best criteria of growth. Objection may be raised to the small number of plants (twelve) used in each group.

For that reason the maximum and minimum dry weights as well as the average are recorded. When the data of the high and low potassium groups of each light condition are examined it will be noted that the minimum of the high group is greater than the maximum of the low potassium group in each of the two cases. There is no overlapping so that the differences in the averages are quite significant. Little doubt can exist that the amount of potassium is the limiting growth factor for plants of groups *OL* and *SL*.

Attention is called to the dry weight ratios of the two groups exposed to the two light conditions.

$$OL/OH \quad 5.2/13.6 = 0.38$$
$$SL/SH \quad 3.6/\ 9.2 = 0.39$$

Here it would appear that light intensity also limits growth, for the ratio of the low to high potassium cultures remains practically the same under two given light conditions. In other words, the low light intensity limits growth

TABLE 2

Average growth of tomato plants grown in solutions of high and low potassium content and under two different light conditions

GROUP	STEM HEIGHT			GREEN WEIGHT (TOPS)			DRY WEIGHT (TOPS)			DRY WEIGHT (ROOTS)
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	cm.	*cm.*	*cm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
OH....................	98	118	70	165	220	104	13.6	17.8	10.4	5.6
OL....................	68	84	53	50	72	30	5.2	6.7	2.9	3.5
SH....................	117	127	93	130	230	72	9.2	16.3	5.3	3.0
SL....................	76	84	57	38	52	28	3.6	4.3	2.3	2.3

in the same proportion whether or not the potassium supply is a limiting factor. This is perhaps better seen when the fractions are rearranged thus:

$$SH/OH = 9.2/13.6 = 0.69$$
$$SL/OL = 3.6/\ 5.2 = 0.69$$

Apparently we have here an example of two limiting factors which are in effect operative at the same time. Considering the plants grown under shade and in a low potassium solution, evidently it was possible to increase their yield by either increasing the potassium concentration of the solution or by improving the condition of illumination, maximum yields being obtained, however, only when both factors were changed. None of the data now available make it possible to suggest an explanation of the mechanism involved in these relations. The actual percentages of potassium in the tissues of the shaded plants were higher than in the corresponding unshaded plants. Perhaps for the production of a given total quantity of dry matter a higher per-

centage of potassium is required with low light values than with high. In any case, experiments of this type have not only theoretical but also practical interest. For example, Russell (13) suggests that there may be a relation between sunshine and potassium fertilization with tomatoes and other plants. Incidentally, in making such comparisons, it is well to note that light values in a greenhouse are much lower than outside, and the quality of the light is also different. It remains to be determined how light values higher than those of the greenhouse, would be related to potassium concentrations in the culture solution.

This first experiment with the flowing solution apparatus clearly showed that a potassium concentration of 3.7 p.p.m. flowing at the rate of 1.2 cc. per minute per plant was below the minimum for the best growth of the tomato under the given conditions. The second experiment, therefore, was modified considerably. The cheesecloth shelter was removed and a single plant was grown

TABLE 3

Average weekly height of tomato plants in experiment 2, together with the maxima and minima of each group

WEEK ENDING	LOW POTASSIUM CONCENTRATION			HIGH POTASSIUM CONCENTRATION		
	Average	Maximum	Minimum	Average	Maximum	Minimum
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
November 4, 1926	2.5	3.1	2.0	2.5	3.0	2.2
November 11, 1926	6.1	7.1	5.4	6.2	6.6	5.5
November 18, 1926	12.5	14.0	11.2	13.0	14.1	11.7
November 25, 1926	27.0	29.4	24.5	27.8	31.2	25.1
December 2, 1926	39.5	44.0	34.5	43.5	52.0	39.2
December 9, 1926	59.1	64.0	54.3	62.7	71.5	57.5
December 16, 1926	84.9	92.6	77.8	91.7	101.8	82.5

in each 5-gallon culture jar. The initial potassium concentrations of the solutions were 3.7 and 35.1 p.p.m. for the *L* and *H* cultures respectively. Each of the two groups contained 12 plants while the rate of flow was 4.6 cc. per minute per plant. The experiment was started October 28 and ended December 16, 1926.

Little, if any, difference existed between the plants of the two groups until November 27, at which time the high potassium plants appeared to be somewhat better in general vigor and their stems slightly thicker. On December 2 measurable differences occurred in the average stem height data, as recorded in table 3.

At time of harvest, the stems of the plants in the high potassium groups were distinctly thicker than those in the low potassium group. Flower buds had developed to some extent in both groups, but none had opened. Slight tip burn appeared on some leaves in both groups while a few leaves on three

of the low potassium plants were somewhat spotted. This particular spotting is characteristic of potassium deficiency in the tomato and resemble that found in the potato under similar conditions. Incidentally it is interesting to note that similar leaf spotting was observed with tomato plants grown in a certain California soil, the displaced solution of which contained potassium in a concentration very similar to that of the low potassium solution used in the present experiments. Figure 1 of plate 2 illustrates this condition of the tomato leaf. The green and dry weights of the plants in the second experiment are given in table 4.

The plants of the two groups in experiment 2 were more nearly alike in size than those of the high and low potassium groups in experiment 1. However, the evidence presented in experiment 2 shows that under these conditions,

TABLE 4

Average green and dry weights of tomato plants in experiment 2, together with the maxima and minima of each group

	LOW POTASSIUM CONCENTRATION			HIGH POTASSIUM CONCENTRATION		
	Average	Maximum	Minimum	Average	Maximum	Minimum
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Green weight						
Tops	110	139	89	209	251	179
Roots	26	33	19	31	35	23
Total	136			240		
Dry weight						
Tops	7.2	9.2	5.7	10.8	13.0	8.5
Roots	1.1	1.4	0.9	1.0	1.2	0.8
Total	8.3			11.8		

3.7 p.p.m. of potassium in a solution flowing at the rate of 4.6 cc. per minute per plant was less efficient than 35.1 p.p.m. of the control solution in producing growth.

A third experiment was carried out similar to experiment 2 but differing only in the rate of flow. The low potassium solutions passed through the culture jars at the rate of 8 cc. per minute per plant. The rate of the high potassium solution was 4 cc. per minute per plant. This experiment was conducted from January 17 to February 21, but even under the increased rate of flow, growth of the tomato plants in the low potassium solution (3.7 p.p.m.) did not equal that of the control plants. Analyses of the residual solution on the last day of the experiment showed only a slight drop in the potassium concentration. The original concentration of potassium was more nearly maintained in this experiment than in any of the others.

Experiment 4 was quite similar to experiment 3 except for one important modification. The same rates of flow were maintained, but the concentration of potassium in the *L* group was increased from 3.7 to 4.9 p.p.m. The experimental period included the time from February 21, to April 7, 1927, and more nearly equalled the length of periods used in the first two experiments. Analyses of the residual solutions on the last day of the experiment showed that the potassium concentrations had been reduced to 2.0 and to 30.6 p.p.m. for the *L* and *H* cultures respectively. The similarity in growth of the plants in these two groups is shown in table 5.

TABLE 5

weekly height and average green and dry weights of tomato plants in experiment 4

WEEK ENDING	LOW POTASSIUM CONCENTRATION	HIGH POTASSIUM CONCENTRATION
	Average weekly height	
	cm.	*cm.*
February 28, 1927	1.9	1.8
March 7, 1927	4.2	4.3
March 14, 1927	8.6	8.5
March 21, 1927	20.0	18.9
March 28, 1927	33.9	31.4
April 4, 1927	46.7	43.5
April 7, 1927	54.8	50.9
	Green weight	
	gm.	*gm.*
Tops	13.3	12.9
Roots	3.0	2.8
Total	16.3	15.7
	Dry weight	
	gm.	*gm.*
Tops	8.8	8.3
Roots	1.4	1.3
Total	10.2	9.6

Under the conditions of these experiments it would appear that the tomato plants required a minimum of approximately 5 p.p.m. initial concentration of potassium when the rate of flow was 8 cc. per minute per plant. In order, however, to test out again the solution of slightly lower concentration flowing at the same rate, experiment 5 was carried out. This was a duplicate of experiment 3, but was conducted at a later date, April 11 to May 23, 1927. The results were similar to those of experiment 3 in that it seemed quite clear that the potassium concentration was below the minimum requirement of the tomato plant. The general appearance of the plants in both sets was very good, but the stems of the high potassium plants were definitely thicker and

the leaves longer than those of the low potassium group. The somewhat stockier appearance of the high potassium plants is indicated in plate 2, figure 2, where a representative plant of each group is shown.

The data from the five experiments on the relation of the minimum potassium level in flowing nutrient solution to the growth of tomato plants are summarized in table 6. With the exception of experiment 3 the durations were approximately equal. The initial potassium concentrations of the solutions are the average values obtained from analyses of the inflowing solutions. During the progress of each experiment, samples of solutions were collected from the plant containers for potassium analysis, but only the analyses of the final samples taken at dates of harvest are given in the table. When the

TABLE 6

Relation of potassium in flowing nutrient solutions to growth of the tomato plant

	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
Period:	*1926*		*1926*		*1927*		*1927*		*1927*	
Beginning	Aug 17		Oct. 28		Jan. 17		Feb. 21		Apr. 11	
Ending	Oct. 5		Dec. 16		Feb. 21		Apr. 7		May 23	
Duration (days)	49		49		35		45		42	
Group	*L*	*H*	*L*	H	*L*	*H*	*L*	*H*	*L*	*H*
Approximate rate of flow per minute per plant *cc.*	1.2	1.2	4.6	4.6	8.0	4.0	8.0	4.0	8.0	4.0
K in solutions										
Initial *p.p.m.*	3.7	35.1	3.7	35.1	3.7	35.1	4.9	35.1	3.7	35.1
Residual *p.p.m.*	0.7	8.5	1.4	23.1	3.5	34.6	2.0	30.6	1.1	24.3
Average height *cm.*	68.0	98.0	84.9	91.7	34.1	38.6	54.8	50.9	49.4	48.1
Dry weight										
Tops *gm.*	5.2	13.6	7.2	10.8	1.8	2.8	8.8	8.3	12.3	19.8
Roots *gm.*	3.5	5.6	1.1	1.0	0.2	0.3	1.4	1.3	1.8	1.9
Total *gm.*	8.7	19.2	8.3	11.8	2.0	3.1	10.2	9.6	14.1	21.7

plants were younger the potassium concentrations were not reduced to the extent they were at time of harvest. The greatest amount of potassium reduction from a concentration of 3.7 p.p.m occurred in experiment 1. Here the rate of flow was lowest. An almost equal reduction occurred in experiment 5, but in this case the rate of flow was much higher. This greater rate of flow should tend to prevent much reduction in the residual solution. However, seasonal conditions were perhaps more conducive to vigorous growth in experiment 5 than in experiment 1 as is indicated by the dry weight data.

The least reduction of potassium from the original concentration of 3.7 p.p.m. occurred in experiment 3. This is not surprising, for the duration of this experiment was only 35 days and in addition the climatic conditions at that time of year were perhaps poorest for growth. These plants were un-

doubtedly less mature than those in the other experiments. This is clearly shown by the dry weights as well as the height measurements.

In experiment 4 the total dry weights of the low and high potassium groups were practically the same. It is therefore reasonable to conclude that a solution originally containing 4.9 p.p.m. potassium flowing at the rate of 8 cc. per minute per plant is as good for growing these tomato plants as is the control solution containing 35.1 p.p.m. potassium flowing at the rate of 4 cc. per minute per plant. The analyses of the final samples collected in this experiment indicate that plants in both the *L* and *H* groups were rapidly reducing the potassium concentrations of their respective solutions.

An interesting observation was made in connection with certain potassium analyses of the solutions collected in experiment 5. The outlet tubes from the culture jars were so arranged that composite samples of the residual solutions could be collected by merely placing empty vessels under the ends of the drain tubes. On May 15, samples were collected at 7.30 a. m. and 4.30 p.m. and on May 22, similar samples were obtained at 9 a. m. and 6.30 p. m. The following potassium concentrations, expressed as parts per million, were found:

	LOW POTASSIUM		HIGH POTASSIUM	
	a.m.	*p.m.*	*a.m.*	*p.m.*
May 15........................	2.24	1.60	28.38	23.88
May 22........................	1.32	0.80	24.40	24.16

In each case, samples collected in the early morning contained more potassium than samples collected in the late afternoon. This is taken to indicate a more rapid absorption of potassium by the plants in the afternoon than in early morning and is probably related to differences in light. Each sample was collected in a 2-quart fruit jar requiring from 30 to 60 minutes. This length of time together with the lag of solution movement in the 5-gallon culture jars would place the exposure periods of the plants well within periods of relatively weak and strong light. If this be true it is in agreement with the work reported by Hoagland and Davis (6) and by Hoagland, Hibbard, and Davis (7) on the absorption of ions as influenced by light.

Chemical data

Chemical analyses were made on the dried plant material from the five experiments. These data, expressed as percentages of water-free material, are presented in table 7. The actual amounts of the elements noted in the table may be determined by multiplying the percentages by the corresponding dry weights noted in table 6.

It will be seen that a very great difference exists between the tops of the plants of the two groups in experiment 1 with respect to the accumulation of certain elements. Accompanying the great depression in the percentage of

potassium found in the L group as compared to that in the H group of plants, there is an increase in the percentage of calcium, magnesium, and phosphate. It will be remembered (see table 6) that in the L cultures of experiment 1, the potassium concentration of the nutrient solution was 3.7 p.p.m. flowing at the rate of 1.2 cc. per minute per plant. In experiment 2 this rate was increased to 4.6 cc. per minute per plant. Here considerable increase in dry weight of the low potassium plants was noted. The percentage of potassium in the plants of the L group in experiment 2 was double that found in experiment 1. Although the percentage of potassium in the H group was also greater than in experiment 1, nevertheless the percentage increase was greater for the L group plants.

TABLE 7

Analyses of tomato plants grown in flowing solutions of low and high potassium concentrations, expressed as percentages of water-free material

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4		EXPERIMENT 5	
Portion of plant analyzed	Tops		Tops		Tops		Tops		Tops	
Group	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*
K	1.94	5.75	3.99	9.08	7.40	9.02	3.68	3.77	3.71	5.90
Ca	3.26	2.25	4.15	3.46	3.77	3.27	3.77	3.50	3.46	2.82
Mg	0.72	0.44	0.95	0.68	0.98	0.84	0.92	0.83	0.80	0.56
N	3.94	3.85	3.98	4.72	6.18	6.33	5.23	5.17	4.41	4.26
PO_4	2.84	1.78	3.71	3.11	3.67	3.21	4.23	4.21	2.79	2.35
Portion of plant analyzed	Roots		Roots		Roots		Roots		Roots	
Group	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*
K	1.12	3.47	1.31	3.05	3.24	6.17	3.57	6.66	1.45	3.47
Ca	1.66	1.88	1.31	1.39	1.44	1.47	1.70	1.45	1.27	1.31
Mg	1.58	0.93	1.62	0.54	1.50	0.60	1.39	1.12	1.72	1.05
N	4.80	5.23	4.89	4.61	5.08	4.79	4.24	4.62	4.95	4.91
PO_4	5.14	4.00	4.98	3.25	7.16	5.79	6.18	5.51	5.56	4.59

Less difference is noted between the percentages of calcium, magnesium, and phosphate of the two groups (L and H) in experiment 2 than in experiment 1. These differences progressively decrease in the order of the first four experiments. The percentages of elements found in the plants of the L and H groups in experiment 4 are more nearly alike than in any of the other experiments. In this experiment the solution rate of flow was 8 cc. per minute per plant and the potassium concentration 4.9 p.p.m. for the L group of plants. Because of the similarity in chemical composition as well as the similarity in general growth appearances of the plants in the L and H groups of experiment 4 it may be concluded that optimum growth can be obtained at a minimum potassium concentration of 4.9 p.p.m. under the conditions of this experi-

ment. It is, of course, realized that there may be other measurements and analyses, which, had they been made, would show the two groups to be quite dissimilar, but with the criteria here used these groups appear identical within the limits of variability and experimental error.

Root data similar to those for the tops of the plants are also given in table 7. In each of the five experiments the percentage of magnesium is higher in the *L* group of plants. Phosphate is also higher in the roots of these plants. Not much difference in the percentage of nitrogen is apparent in the two groups. In this respect there is a similarity between the roots and tops. The roots, however, differ from the tops with respect to their calcium relations since in

TABLE 8

Analyses of tomato plants grown in flowing solutions of low and high potassium concentrations, expressed as milli-equivalents per gram of plant

	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
Portion of plant analyzed	Tops		Tops		Tops		Tops		Tops	
Group	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*
K	0.50	1.47	1.02	2.32	1.89	2.31	0.94	0.96	0.95	1.51
Ca	1.63	1.12	2.07	1.73	1.88	1.63	1.88	1.75	1.73	1.41
Mg	0.59	0.36	0.78	0.56	0.81	0.69	0.76	0.68	0.66	0.46
NO_3	2.81	2.75	2.84	3.37	4.41	4.52	3.73	3.69	3.15	3.04
H_2PO_4	0.30	0.19	0.39	0.33	0.39	0.34	0.45	0.44	0.29	0.25
Sum of bases K, Ca, Mg	2.72	2.95	3.87	4.61	4.58	4.63	3.58	3.39	3.34	3.38
Portion of plant analyzed	Roots		Roots		Roots		Roots		Roots	
Group	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*
K	0.29	0.89	0.34	0.78	0.83	1.58	0.91	1.70	0.37	0.89
Ca	0.83	0.94	0.65	0.69	0.72	0.73	0.85	0.72	0.63	0.65
Mg	1.30	0.76	1.33	0.44	1.23	0.49	1.14	0.92	1.41	0.86
NO_3	3.43	3.73	3.49	3.29	3.63	3.42	3.03	3.30	3.53	3.50
H_2PO_4	0.54	0.42	0.52	0.34	0.75	0.61	0.65	0.58	0.59	0.48
Sum of bases K, Ca, Mg	2.42	2.59	2.32	1.91	2.78	2.80	2.90	3.34	2.41	2.40

both the *L* and *H* groups the percentages of calcium contained in the roots are very similar, but this is not true of the tops.

In order to show to what extent the actual chemical units replace each other, the data given in table 7 have been calculated to milliequivalents per gram of dry plant material. These values are set forth in table 8.

The milliequivalents of potassium in the plant tops of the *L* groups of experiments 1, 2, 3 and 5, are appreciably less than those of the corresponding *H* groups. On the other hand the milliequivalents of calcium and magnesium are higher in the *L* groups. The sums of the milliequivalents of potassium, calcium, and magnesium were determined for each group and the percentages of potassium based on these sums are as follows:

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4		EXPERIMENT 5	
	L	*H*	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*
Sum	2.72	2.95	3.87	4.61	4.58	4.63	3.58	3.39	3.34	3.38
Per cent	18	50	26	50	41	50	26	28	28	45

It is rather remarkable that such a constant relationship (approximate value of 50 per cent) exists between potassium and the other two elements in all of the *H* groups, except that of experiment 4. This relationship is especially interesting since the plants had been grown at different times of the year. Just why the plants in experiment 4 were an exception is not known. Both groups in this experiment had practically the same value. From these data it appears that the plants grown in potassium-deficient solutions tend to accumulate a greater amount of calcium and magnesium than when they are fully supplied with potassium. The replacement of calcium and magnesium in the plant tops by potassium is perhaps better seen when the *increase* of calcium and magnesium in the *L* groups over that found in the *H* groups is compared with the *decrease* of potassium in the *L* groups below that in the *H* groups.

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3	EXPERIMENT 4	EXPERIMENT 5
Ca and Mg increase	74	56	37	21	52
K decrease	97	130	42	2	56

It is also desirable to consider the data from the point of view of the total number of milliequivalents of bases (K, Ca, Mg) contained in the plants of the *H* and *L* groups (table 8). In both the roots and tops there is evident a marked tendency for a decrease in potassium to be compensated by a chemically equivalent amount of calcium and magnesium. Thus the relative proportions of the different bases are subject to more radical alteration than is the total base content of the plant. This may be important as bearing on the internal equilibrium between mono- and divalent cations.

The total number of grams of potassium per plant (expressed separately for tops, roots, and total) is presented in table 9. These data are compared with the potassium concentrations of the solutions surrounding the plant roots. The volume of the plant containers was approximately 5 gallons each. The total maximum number of grams of potassium to which the roots were exposed at any one moment is given in the last line of the table. In reality the actual values were perhaps below these figures, especially when the plants were well grown.

It will be noted that the number of grams of potassium found in the roots was never greater than that in the total volume of solution surrounding them. On the other hand the number of grams found in the tops was always greater

than that found in the nutrient solutions, with two exceptions, the *H* groups in experiments 3 and 4. It is especially interesting to note there was more potassium in the tops of all the *L* groups than in the solutions surrounding their roots. While more total potassium was taken up by the *H* group plants than by the *L* group plants, the ratio of the amounts absorbed by the *L* groups to the concentration of their nutrient solutions was far greater than in the case of the *H* group plants.

If it be assumed that nearly all of the potassium found in the roots and tops was present in the cell sap in dissolved state, then the concentration of potassium in the sap must have been from a hundred to a thousand times greater than in the culture solution which bathed the root system. The factor of concentration (concentration in sap divided by concentration in solution) would be far higher for the solution of low concentration than for the one of high concentration. The relations are consistent with experiments on other types of agricultural plants and on certain aquatic plants, such as Nitella.

TABLE 9

Total grams of potassium per plant compared with the possible maximum potassium concentration of the nutrient solutions surrounding the plant roots at any given time

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4		EXPERIMENT 5	
Group	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*	*L*	*H*
Tops	0.101	0.782	0.287	0.981	0.133	0.253	0.324	0.313	0.456	1.168
Roots	0.039	0.194	0.014	0.031	0.003	0.019	0.050	0.087	0.026	0.066
Total	0.140	0.976	0.301	1.012	0.136	0.272	0.374	0.400	0.482	1.234
Solution concentration p.p.m. K	3.7	35.1	3.7	35.1	3.7	35.1	4.9	35.1	3.7	35.1
Approximate gm. K in 5 gallons	0.070	0.664	0.070	0.664	0.070	0.664	0.093	0.664	0.070	0.664

The chemical data here presented indicate that a potassium concentration of approximately 5 p.p.m. in the nutrient solution flowing at the rate of 8 cc. per minute per plant was exactly at, or slightly below, the minimum required by the tomato plants in these experiments. A slightly greater concentration or a slightly greater rate of flow would, in all probability, merely result in a somewhat higher concentration of potassium in the root sap. So far as can be seen at present, such an increase would be of little or no benefit to the plants, at least for the stages of growth under observation.

DISCUSSION

The main purpose of the five experiments here reported was to approximate a minimum potassium concentration necessary for optimum growth of the tomato plant. The effect of volume or total supply of nutrients is a serious handicap in any study of this sort. Stiles (16) emphasizes this matter in his statement, "The extent to which concentration of the nutrient solution can

act as a limiting factor is difficult to examine, because from the moment the plant roots are put in the solution the concentration will be altering. It was in an attempt to minimize this difficulty that the writer changed the water culture solutions at frequent intervals. Even then the concentration of the nutrient solution will only remain very approximately constant when the rate of growth is slow." Hoagland (4) voices a similar criticism in his discussion of several salt nutrition studies in which sufficient distinction was not made between "supply of nutrients and concentration of nutrients." A number of investigators have made use of large volumes of solution while others have changed the plant nutrient solutions frequently in order to maintain a fairly constant concentration and to reduce reaction changes to a minimum. It is obvious, however, that the use of rapidly flowing solutions more nearly fulfills the requirements for studies of minimum salt level than any other method so far devised. In pointing out the importance of continuous renewal of nutrient solutions for plants, Trelease and Livingston (17) state, "If the rate is rapid enough, the discarded solution will not be significantly different from the inflow, and the roots may be said to have been in a known set of chemical surroundings throughout the culture period."

One further point should be mentioned in any critical review of experiments with flowing culture solutions. The determinations of concentration are made on samples representing the main body of the solution in which the root system is immersed. The concentration of any element in the films of solution surrounding the absorbing root membranes is unknown. The degree to which a given concentration is maintained in these films may depend in part on the thoroughness with which the solution is kept mixed mechanically, by rapid flow of solution or by some other form of stirring.

It is not necessary at this time to enter into a discussion of the interpretation of solution culture data in terms of soil problems, but it seems desirable to mention the point that it is possible that plants growing in soil or sand may develop a much greater total absorbing root surface than in a culture solution, thus providing to a greater or lesser extent a means of compensation for low soil solution concentrations. On the other hand, earlier comparisons of solution and sand cultures each involving the same total amounts of nutrient, showed that barley plants accumulated greater percentages and amounts of mineral elements from the solution condition, notwithstanding the greater root development occurring in the sand cultures. Presumably these effects were explained by the ease of diffusion (or mixing by convection currents) in the solution cultures. However, the comparison of sand and solution cultures is not the same as that of soil and solution cultures, since the soil has a "supplying" power of its own. We have then, entering into the equation, in addition to concentration effects, relative areas of absorbing root surfaces, rates of diffusion or of mixing in culture solutions, and rates of solution of solid components in the soil. Unfortunately we have no means of evaluating certain of these factors, and therefore we cannot make any accurate comparison between soil solutions and artificial culture solutions.

Both the growth data and the chemical analyses of the plants and solutions in the five experiments reported in this paper indicate the minimum low level of potassium concentration to be approximately 5 p.p.m. for the tomato plants grown under the climatic and other conditions of the experiments. This is in good agreement, as to general magnitude involved, with results reported by the Arkansas Experiment Station (1) in determining the minimum amount of potassium needed for plant growth. These results led to the conclusion that between 3 and 5 p.p.m. potassium seemed sufficient for best growth of the tomato. Parker and Pierre (12) in their work at Alabama with corn and soybeans varied the potassium concentrations of their nutrient solutions from 0.5 to 25 p.p.m. Although maximum growth of corn was obtained in the solution of 10 p.p.m. and that of soybean in the solution of 25 p.p.m., nevertheless the growth obtained in solutions of 2 p.p.m. potassium was practically as good. These authors believe that the results obtained show that both plants will make maximum growth at potassium concentrations of 2 p.p.m. or possibly less. In comparing results obtained by different investigators it is desirable to emphasize once more the necessity for keeping in mind climatic conditions, variety of plant, and significance of data on basis of plant variability. It is not to be expected that exact and fixed values will ever be established but only that a better understanding will be obtained of the magnitudes involved.

It is not the purpose of the present paper to outline the rôle of potassium in the physiology of the tomato plant, but the influence potassium plays in its effect on the absorption of other elements cannot be overlooked. It has been known that one ion frequently influences the absorption of other ions and that sodium may be substituted for potassium to a certain extent. Experiments with barley (5) at the California station, clearly suggest that sodium and potassium ions may exert a depressing effect on the absorption of calcium ions. The interrelations of potassium, calcium, and magnesium as indicated in these experiments are consistent with those found in experiments of various types made on different plants in California. These experiments include studies made on citrus and walnut seedlings by Haas and Reed (3). Ginsburg (2) working with soybean plants grown in culture solutions each lacking a definite element, found the average composition (expressed as percentage) of the plants deficient in potassium to be the following as compared with plants grown in a complete solution:

	ASH	Ca	Mg	N
Complete solution	13.31	1.34	1.10	3.22
Potassium-deficient solution	12.69	3.15	1.15	4.55

Here an increase of calcium is noted in the potassium-deficient cultures. These observations are in general agreement with the tomato experiments in that the amounts of calcium absorbed decreased as the amounts of potassium increased, but no agreement was found regarding the magnesium and

nitrogen relations. With the tomato, little if any relation was manifest between the potassium and nitrogen content of the plants, the percentage of nitrogen remaining quite constant in all five experiments. No direct comparison can be made, however, for in Ginsburg's experiments potassium was completely lacking, and, furthermore, soybean plants were used.

The spotting of tomato leaves was very characteristic of plants grown in potassium-deficient solutions. These spots were dark brown and appeared first in the older leaves. This condition occurred only where the potassium supply was extremely low. Similar symptoms of "potash hunger" have been observed in other plants such as the potato, cotton, buckwheat and bean. Regarding "potash hunger" symptoms, Sorauer (15) states, "At first near the leaf edges and then later scattered over the whole surface of the leaf, appear yellowish spots which rapidly turn brown or often change to white, while the petioles and veins together with the immediately adjacent tissues remain green. Finally the leaves dry up, beginning usually at the edges, with a dark brown color." This describes very well the conditions occurring in the tomato plant when the potassium supply becomes greatly depleted. These conditions were more frequently observed in cultures of other experiments where the solutions were not changed. However, in this series of experiments, where the potassium concentration was low, and the rate of flow slow, the spotting appeared. This peculiar leaf reaction gives promise of being a reliable index of potassium deficiency of tomato plants grown in nutrient media.

SUMMARY

Concentration and volume are two distinct characteristics of a plant nutrient solution. In order to determine the minimum concentration of a given element that will produce optimum growth, the volume of the solution or total supply should be sufficiently large that it will not become the limiting factor. With a newly devised flowing solution apparatus, experiments were undertaken to determine the minimum potassium level requirement of the tomato plant. Under the given experimental conditions, which were kept within a range for good growth, it was found that optimum growth was maintained at a potassium concentration of approximately 5 p.p.m. at the intake. The rate of flow for such a solution averaged 8 cc. per minute per plant. The plants were grown for a period of 45 days. In experiments where the initial potassium concentration of 3.7 p.p.m. was reduced to 0.7 p.p.m. or even to 1.4 p.p.m., actual analysis of the plants showed a marked decrease in potassium absorbed and a tendency toward increased calcium, magnesium, and phosphate absorption as compared with check plants. A characteristic spotting of leaves indicating "potash hunger" is very marked in tomato plants grown in solutions in which the potassium concentration is maintained below a certain level. Attention is called to certain interesting relations between potassium concentrations and light values, as suggested by one of the experiments.

REFERENCES

(1) Agronomy Department. 1927 The rôle of potassium in plant nutrition. *Ark. Agr. Exp. Sta. Bul.* 221: 63.

(2) Ginsburg, J. M. 1925 Composition and appearance of soybean plants grown in culture solutions each lacking a definite essential element. *Soil Sci.* 20: 1–13.

(3) Haas, A. R. C., and Reed, H. S. 1926 The absorption of ions by citrus and walnut seedlings. *Hilgardia* 2: 67–106.

(4) Hoagland, D. R. 1919 Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. *Jour. Agr. Res.* 18: 73–117.

(5) Hoagland, D. R. 1923 The absorption of ions by plants. *Soil Sci.* 16: 225–246.

(6) Hoagland, D. R., and Davis, A. R. 1923 Further experiments on the absorption of ions by plants, including observations on the effect of light. *Jour. Gen. Physiol.* 4: 47–62.

(7) Hoagland, D. R., Hibbard, P. L., and Davis, A. R. 1926 The influence of light, temperature, and other conditions on the ability of Nitella cells to concentrate halogens in the cell sap. Jour. Gen. Physiol. 10: 121–146.

(8) Johnston, E. S. 1921 The seasonal march of the climatic conditions of a greenhouse, as related to plant growth. Md. Agr. Exp. Sta. Bul. 245.

(9) Johnston, E. S. 1927 An apparatus for controlling the flow of nutrient solutions in plant cultures. *Plant Physiol.* 2: 213–215.

(10) Johnston, E. S. and Dore, W. H. 1928 The relation of boron to the growth of the tomato plant. *Science* 67: 324–325.

(11) Johnston, E. S. and Dore, W. H. The influence of boron on the chemical composition and growth of the tomato plant. *Plant Physiol.* (In press.)

(12) Parker, F. W., and Pierre, W. H. 1928 The relation between the concentration of mineral elements in a culture medium and the absorption and utilization of those elements by plants. *Soil Sci.* 25: 337–343.

(13) Russell, E. J. 1927 Soil conditions and plant growth, p. 84–88. New York.

(14) Shive, J. W., and Stahl, A. L. 1927 Constant rates of continuous solution renewal for plants in water cultures. *Bot. Gaz.* 84: 317–323.

(15) Sorauer, P. 1915 Manual of Plant Diseases. Translated by Frances Dorrance. Wilkes-Barre, Pa.

(16) Stiles, W. 1916 On the interpretation of the results of water culture experiments. *Ann. Bot.* 30: 427–436.

(17) Trelease, S. F., and Livingston, B. E. 1922 Continuous renewal of nutrient solution for plants in water cultures. *Science* 55: 483–486.

PLATE 1

Fig. 1. Apparatus for controlling the flow of nutrient solutions.

Fig. 2. Tomato plants of the unshaded high and low potassium groups of experiment 1, together with the cheese cloth shelter containing the shaded groups of plants.

Fig. 1

Fig. 2

PLATE 2

FIG. 1. Spotting of tomato leaf (left) resulting from potassium deficiency, compared with normal leaf (right).

FIG. 2. Representative plants of low (3.7 p.p.m.) and high (35.1 p.p.m.) potassium cultures of experiment 5.

FIG. 1

FIG. 2

THE MEASUREMENT OF "SUCTION FORCES" IN SOILS

CHAS. F. SHAW

California Agricultural Experiment Station

Received for publication October 1, 1928

In an endeavor to measure the force exerted by soils in absorbing or imbibing water, Joffe and McLean (2, 3) used a porous burned clay cup (Pasteur clay filter or filter balloon) filled with water and connected to a mercury manometer. The soil was packed around the porous cup, and as the water was absorbed by the soil, the mercury was lifted until its weight balanced the "suction" force that was developed. The method has been suggested as a means of indirectly measuring the colloidal content of the soils. Hardy (1) has criticized the method on the grounds that it does not take into account the variation in water conductivity of the soils.

In experiments similar to those of Joffe and McLean, we have been unable to get results that show any significant differences between soils of markedly different soil texture. The final results, as measured by the height of the column of mercury, are approximately the same for all the soils tested.

The porous "cups" used were Pasteur-Chamberlain filter thimbles, porous irrigation tubes, and thistle top atmometers. In some experiments the tubes or cups were packed in soil and attached to the usual type of U-shaped mercury manometer; in others, they were placed in percolation cylinders, the connecting glass tubing extending vertically downward into the mercury reservoir; while in some experiments the thistle top atmometers were fitted with collars and the soil was packed upon their upper surface, with a straight tube to the mercury reservoir (fig. 1). Table 1 shows the results from a typical experiment by the latter method. The soils used were Tujunga sand with a total clay content of 2.57 per cent, of which 0.83 per cent was colloidal clay (less than 2 μ effective diameter); Hanford fine sandy loam with 8.35 per cent total clay and 4.59 per cent colloidal clay; and Vina clay loam with 24.73 per cent total clay and 16.35 per cent colloidal clay. In this particular experiment, the atmometers had been soaked and boiled in distilled water to drive out included air and were covered with a uniform depth of 2 inches of soil packed on the absorption surface. Table 1 and curve 4 on the graph (fig. 2) show that the rate and height of rise of the mercury was relatively uniform for each soil, notwithstanding their great differences in texture.

Parallel tests were carried out, a similar atmometer cup, without any soil, being used. The results are shown in table 2 and as curves 1, 2, and 3 on the graph (fig. 2). In this case the rate of rise was much faster and the total

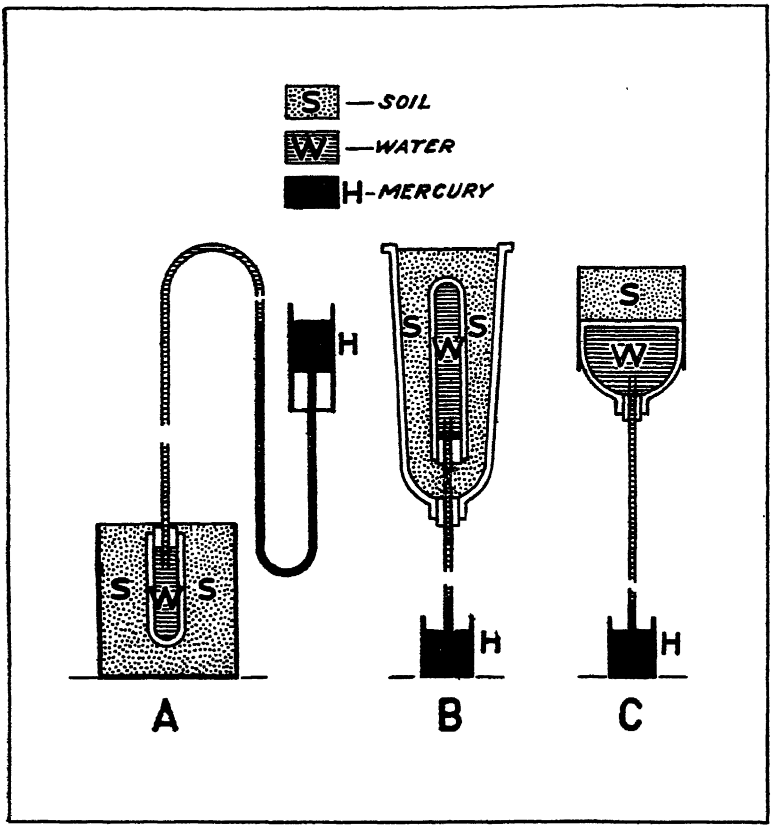


FIG. 1. METHODS OF SETTING UP THE EXPERIMENTS

A—tube packed in soil with usual type mercury manometer. *B*—tube packed in soil in a percolator, with straight vertical glass tube extending into mercury reservoir. *C*—tube (atmometer) with soil packed on its surface, and with straight glass tube to mercury reservoir.

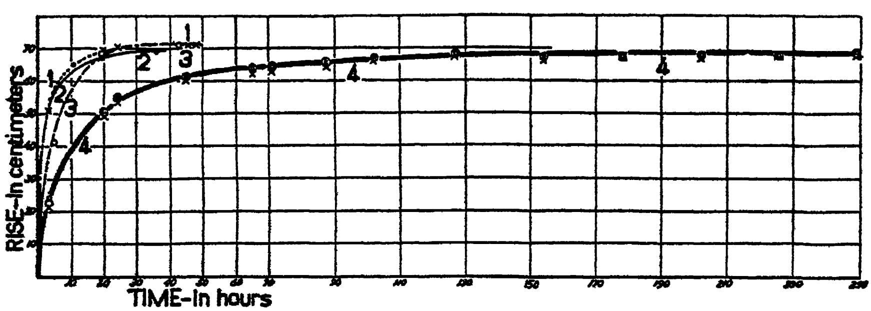


FIG. 2. RATE AND HEIGHT OF RISE OF MERCURY LIFTED BY ATMOMETER CUPS FILLE WITH WATER

Curves 1, 2, 3 are successive tests with surface of cup exposed to evaporation. Curve 4 is drawn through points on three tests using Tujunga sand, Hanford sandy loam, and Vina clay loam.

TABLE 1

Height to which mercury was lifted by porous atmometer cups filled with water and covered with two inches of soil

TIME ELAPSED	HEIGHT OF MERCURY		
	Tujunga sand	Hanford sandy loam	Vina clay loam
hours	*cm.*	*cm.*	*cm.*
0.50	9.00	10.00	11.00
0.83	13.75	12.25	13.75
2.25	22.25	20.00	21.75
2.50	24.00	21.30	23.30
18.90	50.60	49.20	50.80
20.95	53.40	51.50	53.00
22.00	54.30	52.50	53.90
23.50	55.30	53.70	55.00
46.50	60.10	61.30	61.90
47.50	60.30	61.40	62.20
48.75	60.70	61.90	62.60
65.00	62.30	64.00	64.00
67.00	62.60	64.20	64.30
70.00	62.80	64.30	64.50
71.00	62.90	64.50	64.60
87.50	64.60	66.10	66.10
93.00	64.60	66.10	65.90
95.00	64.60	66.10	65.90
102.00	66.00	67.20	67.10
103.50	66.00	67.50	67.15
110.50	66.00	67.60	67.15
127.00	66.60	68.10	67.80
154.00	66.50	67.80	67.50
165.50	67.00	68.40	68.00
178.50	67.00	68.30	67.80
181.00	67.00	68.30	67.80
202.50	67.25	68.60	68.10
222.00	67.80	69.20	68.60
226.75	67.60	69.10	68.40
231.00	67.70	69.20	68.50
246.00	68.00	69.30	68.60
249.00	67.75	69.20	68.60
269.75	67.90	69.40	68.80
293.50	67.70	Dropped	Dropped
298.75	67.60	.....	.....
302.00	67.50	.....	.....
334.00	67.30	.....	.....
342.00	67.20	.....	.....
356.00	Dropped	.....	.....

height somewhat greater than that obtained when the atmometer cups were packed with soil! The lifting force, or "suction" evidently rests in the porous burned clay disc that forms the surface of the atmometer, and not in the soil.

This agrees with the statements of Livingston (4) and others that the "suction" force developed by a clay cup may exceed one atmosphere.

It is our belief that the experiment does not measure the "suction" force of the soil, but that it may to a degree measure the ability of the soil to transport moisture away from the absorption surface. In the experiment shown in table 1 the moisture evaporated from the soil nearly as fast as the atmometer cup could deliver it, and continued until the weight of the mercury caused air

TABLE 2

Height to which mercury was lifted by porous atmometer cups filled with water and exposed to evaporation in laboratory

Columns 1, 2, and 3 show results from three successive tests, the atmometer cups being boiled in distilled water between tests.

TIME ELAPSED	HEIGHT OF MERCURY		
	1	2	3
hours	*mm.*	*mm.*	*mm.*
0.50	21.5	....	....
0.83	21.5	....	....
2.25	48.5	....	....
2.50	51.5	....	....
4.00	....	....	42.1
11.00	....	66.0	....
12.25	....	66.0	....
18.90	69.7	....	....
19.00	....	....	68.9
19.50	....	67.1	....
20.95	70.2	....	....
22.00	70.3	....	69.0
23.50	70.3	....	....
36.00	....	69.1	....
39.00	....	Dropped	....
43.00	....	....	71.0
46.50	71.3	....	....
47.50	71.3	....	....
48.75	71.4	....	....
65.00	Dropped	....	....
66.75	....	....	Dropped

to be sucked into the interior cavity of the atmometer. The air moved in slowly (as shown by the lowering of the mercury columns of the sand tube) at first, but as the air bubble enlarged, the mercury receded at an accelerating rate until it finally dropped.

CONCLUSIONS

The "suction" force of soils, and indirectly the colloid content, can not be measured by the use of porous burned clay or porcelain cups, bulbs, or tubes,

because these porous materials themselves have a high water lifting or "suction" force. The soil may serve to remove the moisture from the surface of the material and thus make possible a continuation of the process until the weight of the mercury lifted overcomes the tensile strength of the water column or the forces which hold the water in the pores of the "cup" material. The soil, however, would not carry any of the weight, nor supply any of the "suction" force. It would function essentially the same as though it were lifting water from a free water surface and would develop a water content essentially the same as that of the capillary rise.

REFERENCES

(1) HARDY, F. 1927 The measurement of "suction forces" in colloidal soils. *Soil Sci.* 24: 71–75.

(2) JOFFE, J. S., AND MCLEAN, H. C. 1925 Colloidal behavior of soils and soil fertility: I. Suction force of soils as an index of their colloid content. *Soil Sci.* 20: 169–174.

(3) JOFFE, J. S., AND MCLEAN, H. C. 1926 Colloidal behavior of soils and soil fertility. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1925: 305–308.

(4) LIVINGSTON, B. E., AND LUBIN, GRACE. 1927 The Askenasy demonstration of traction transmitted through liquid water. *Science* 65: 376–379.

SOME EFFECTS OF CRUDE PETROLEUM ON NITRATE PRODUCTION, SEED GERMINATION, AND GROWTH[1]

H F. MURPHY[2]

Oklahoma Agricultural and Mechanical College

Received for publication October 6, 1928

The effect of crude petroleum on soils is of practical importance only in the oil field districts and along pipe lines where breaks may occur and allow the crude petroleum to overflow the nearby land. The question of damages under such conditions is continually occurring. Also, after the oil industry in a particular section has given out, the question of bringing the land back to the production of field crops makes the effect of crude petroleum on land an important problem in such cases.

Literature on the effect crude petroleum may have on the soil and further crop production is meager. Several investigators have shown that certain soil organisms have the power to utilize hydrocarbons as sources of energy yet few of these workers have used crude petroleum as the source of hydrocarbons under consideration.

Rahn (4) in 1906 reported a mold found in soils that was capable of breaking down paraffin and utilizing it as a source of food. Söhngen (5) in 1913 reported several organisms that could readily utilize hydrocarbons as sources of energy. By using a special medium, he found that 4 to 8 mgm. of petroleum were oxidized in 24 hours at 28°C. for every square centimeter of surface of solution. Gainey (3) reported that paraffin was detrimental to ammonification and nitrification. Tausz and Peter (6) reported in 1919 some isolated soil organisms capable of decomposing hydrocarbons. They isolated the three organisms *Bact. aliphaticum*, *Bact. aliph. liquefaciens*, and paraffinbacterium which were capable of decomposing hydrocarbons. Carr (2) found that the growth of soybeans was apparently improved when a small amount of oil (up to 0.75 per cent) was added to soil and that a rather large amount (4 per cent) could be added before the soybean plants succumbed to the treatment. Baldwin (1), in studying the modification of soil flora induced by applying crude petroleum, found that the soil flora was considerably changed. Ammonia production was slightly lowered and nitrate production was completely inhibited over varying periods of time. Small applications of crude petroleum, however, did not seem to injure the crop producing power of the soil.

[1] Contribution from the Department of Field Crops and Soils.

[2] Associate Professor of Field Crops and Soils.

EXPERIMENTAL WORK

The experimental work carried on by the writer was divided into two parts: part 1 consisted of studying the effect of crude petroleum on nitrification, whereas Part 2 had to do with the effect of petroleum on seed germination and plant growth. The soil used in both parts is what was classified by the U. S. Department of Agriculture, Bureau of Soils, as "Yahola very fine sandy loam." This is a common bottomland soil found along the streams in the Red Prairie division of the Great Plains region. It is considered a very productive soil in the section where it is found.

TABLE 1

Nitrate content of soils incubated with various amounts of crude petroleum applied in

AMOUNT OF CRUDE PETROLEUM APPLIED	TREATMENT	OCTOBER	NOVEMBER	JANUARY	FEBRUARY
per cent		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0	None	132	83	45	56
0.0843	Surface	....*	70	46	41
0.0843	Mixed	....	55	53	37
0.2107	Surface	....	39	44	32
0.2107	Mixed	....	....	....	17
0.4215	Surface	66	34	29	28
0.4215	Mixed	49	8	15	11
0.6322	Surface	....	25	42	15
0.6322	Mixed	....	....	....	8
1.0959	Surface	63	4	28	9
1.0959	Mixed	10	0‡	5	6
2.1918	Surface	16	0‡	10	4
2.1918	Mixed	3	0	0	5
6.5754	Surface	Trace†	0	0	....
6.5754	Mixed	Trace	Trace	0	....

* A blank indicates no sample was run for that period.
† None in one of the duplicates.
‡ One of the duplicates showed a very slight trace.

Part 1

In the nitrification study, 100-gm. samples of soil were placed in glass tumblers and a definite amount of crude petroleum that had a specific gravity of 0.8430 was added. The crude petroleum was used in one of two ways; either it was applied to the surface or it was thoroughly mixed with the soil. The samples were kept at optimum moisture conditions and incubated under greenhouse conditions for one month. At the end of the month, the nitrates were determined by the phenoldisulfonic acid method. Very good checks were secured. The results of these nitrification tests are given in table 1. The tests covered a period of four months, new sets being incubated each month.

The results indicate that even a very small application has a tendency to

reduce nitrate formation. There was very little difference between mixing the crude petroleum with the soil and applying it very lightly on the surface, but as the rate of application increased, mixing the crude petroleum with the soil decreased nitrate production more than did surface application. The application of approximately 0.4 per cent of crude petroleum to the surface cut down the nitrates about one-half, whereas approximately 1 per cent of crude petroleum mixed with the soil practically checked nitrate formation. Even smaller amounts of crude petroleum when mixed with the soil had a very depressing effect on nitrate production. The application of crude petroleum amounting to approximately 0.4 per cent when mixed with the soil very materially checked the formation of nitrates.

TABLE 2

Effect of crude petroleum on the germination and growth of wheat

TREATMENT (ACRE BASIS)	NUMBER OF PLANTS PER PLOT				
	September 24	September 27	September 29	October 5	October 17
No treatment	4	8	22	22	22
15,000 gallons applied to surface	None	None	None	None	None
5,000 gallons applied to surface	None	None	2	5	5
2,500 gallons applied to surface	None	None	2	14	25
500 gallons applied to surface	None	1	22	25	25
15,000 gallons applied at 4-inch depth	None	None	None	None	None
5,000 gallons applied at 4-inch depth	None	1	2	19	22
2,500 gallons applied at 4-inch depth	None	1	20	25	25
15,000 gallons mixed with surface 4 inches	None	None	None	None	None
5,000 gallons mixed with surface 4 inches	None	None	None	None	None
2,500 gallons mixed with surface 4 inches	None	None	None	1	1
500 gallons mixed with surface 4 inches	None	2	7	16	16

Part 2

The effect which crude petroleum has on the germination of the wheat seed and on the growth of wheat was studied on 8½ pounds of soil placed in one-gallon tin containers. These soil samples were treated with various amounts of crude petroleum added in different ways. The cans were buried in the ground so that the surface of the soil in the container was even with the surface of the soil on the outside. The bottom of each container was supplied with drainage holes. Moisture for the germination of the seeds and for the development of the seedlings was supplied by the rainfall during the fall season of 1927. The crude petroleum was added in the following ways to various samples as noted in table 2: (*a*) poured on the surface, (*b*) four inches of soil was removed from the container, the petroleum poured over the surface of the remaining soil in the container, and the top soil put back in place, and (*c*) the crude petroleum was mixed with the surface four inches of soil. Twenty-five kernels of Turkey Red wheat were planted about one and one-half inches

deep in each container on September 9. The first rain after the wheat was planted occurred on September 23, when 1.34 inches fell. Table 2 gives the result of the germination and growth experiment.

Crude petroleum, even in small amounts, generally delayed germination. The containers in which the seed did not germinate were examined on October 19, and it was found that all of the wheat kernels had rotted. Mixing the crude petroleum with the surface was noticeably detrimental to seed germination. Five hundred gallons of crude petroleum mixed with the surface 4 inches of soil reduced the stand 36 per cent compared with the check average on October 17. Twenty-five hundred gallons of crude petroleum applied to the surface gave only approximately 23 per cent of a total stand compared with the check average on October 17. A similar amount of crude petroleum applied 4 inches below the surface did not reduce the final stand on October 17. Mixing any of the larger applications of crude petroleum with the surface soil prevented germination of the seed. Five hundred gallons applied to the surface did not prevent germination but did delay it just slightly, as can be noted under the column dated September 27.

REFERENCES

(1) Baldwin, I. L. 1922 Modifications of the soil flora induced by applications of crude petroleum. *Soil Sci.* 14: 465–478.

(2) Carr, R. N. 1919 Vegetative growth in soils containing crude petroleum. *Soil Sci.* 8: 67.

(3) Gainey, P. L. 1917 Effect of paraffin on the accumulation of ammonia and nitrates in the soil. *Jour. Agr. Res.* 10: 355.

(4) Rahn, O. 1906 Ein paraffin zerstezender Schimmelpilz. *Centbl. Bakt.* (2) 16: 382. (Original article not read.)

(5) Söhngen, N. L. 1913 Benzin, Petroleum, Paraffinol, und Paraffin als Kohlenstoff- und Energiequelle für Mikroben. *Centbl. Bakt.* (2) 37: 595–609. (Original article not read.)

(6) Tausz, J., and Peter, M. 1919 Neue Methode der Kohlenwasserstoffanalyze mit Hilfe von Bakterien. *Centbl. Bakt.* (2) 49: 497–554. (Original article not read.)

DETERMINATION OF THE FINENESS OF MARL[1]

E. P. DEATRICK AND CLARENCE DORMAN[2]

West Virginia Agricultural Experiment Station

Received for publication October 19, 1928

The accuracy of the standard method of determining the fineness of marl has been questioned alike by some marl producers and control chemists. Data are herewith presented to show that determinations made according to the standard method—that of calculating the percentages of material of certain finenesses from the weights of the thoroughly dried material held on the screens after thorough shaking—is inaccurate.

It is not reasonable to expect that dry screening would yield accurate results, for the large pieces of marl are not solid, but are balls or granules of finer particles which to a great extent have been formed during the commercial drying process. Apparently recognizing this, different laboratories seem to vary the details of the method of screening.

In one laboratory the marl is gently rubbed through the screens with a rubber pestle. In another the marl is constantly agitated with a spoon handle or similar instrument, but no attempt is made to "force the particles through the mesh or break them down in any way."

It is exceedingly difficult to agitate so that only aggregates are broken up and so that particles are not actually forced through the mesh. Inasmuch as marl closely resembles soil, it would seem natural that the use of the principle employed in determining the fineness of soil would yield more accurate results. Here the sample is deflocculated before screening is attempted.

By screening the marl after it is deflocculated, it has been found, as shown in table 1, that the material is really very much finer than the dry screening test would indicate. The amount of material passing a 0.1-mm. screen is in some cases 70 per cent greater after deflocculation. The deflocculation is conveniently accomplished by placing the dry marl (in this case 10 gm.) in a motor drink mixer as modified by Bonyoucos[3] and allowing it to run for 9 minutes (the time required to deflocculate completely most soils) after 500 cc. of water and 5 cc. of 0.1 N KOH have been added. After the samples were

[1] Approved as scientific paper no. 64 by the director. Read before the Fertilizer Section of the American Chemical Society, September, 1928.

[2] Associate Professor, Agronomy (Soils) and Graduate Student Assistant, W. Va. Agricultural Experiment Station, Morgantown.

[3] Bonyoucos, G. J. 1927 Directions for determining the colloidal material of soils by the hydrameter method. *Science* 66 (1696): 16.

deflocculated, the suspensions were poured through a battery of soil sieves of 1-, 0.5-, 0.25-, and 0.1-mm. mesh. These were used instead of the customary 20-, 40-, 60-, 80-, and 100-inch mesh sieves because those in our laboratory are too large to put on the pan of the analytical balance. It was found later that the deflocculated marl should be poured on a battery of the 1-, 0.05-, and 0.25-mm. sieves first. The collected water with the material finer than 0.25 mm. should then be poured separately on the 0.1-mm. screen with constant agitation with a rubber policeman so as to prevent clogging of the fine meshes. In this way the determination can be carried out easily and accurately.

TABLE 1

Mechanical analysis of marl

NAME OF MARL	SIZE OF MATERIALS IN MILLIMETERS, DETERMINED BY									
	Dry screening					Wet screening				
	1	0.5	0.25	0.1	>0.1	1	0.5	0.25	0.1	>0.1
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lime marl	10.0	17.5	13.7	46.7	12.1	0.3	0.7	2.9	12.3	83.8
Precipitated lime	0.0	7.6	18.2	63.3	11.0	0.0	4.2	12.5	25.8	57.5
Alba marl	32.5	22.3	16.9	21.5	6.8	0.5	1.0	4.6	9.9	84.0

TABLE 2

Mechanical analysis of ground limestone

NAME OF LIMESTONE	SIZE OF MATERIALS IN MILLIMETERS DETERMINED BY									
	Dry screening					Wet screening				
	1	0.5	0.25	0.1	>0.1	1	0.5	0.25	0.1	>0.1
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Standard limestone	0.6	8.5	20.0	42.6	28.3	0.0	5.5	15.6	24.0	54.9

For those specially interested in this work, it may be stated that screening of 20-, 40-, 60-, 80-, and 100-inch mesh can easily be secured and inserted in sieve holders such as were used here (no. 12110 from Central Scientific Company).

It will be noticed that, as shown in table 2, dry screening of ground limestone does not completely separate out the material of finest size, though, as expected, the difference between the two methods of screening is not so great as in the case of marl.

EFFECT OF CROP GROWTH ON THE REPLACEABLE BASES IN SOME CALIFORNIAN SOILS

J. C. MARTIN

University of California[1]

Received for publication October 29, 1928

In 1915 there was inaugurated at this laboratory an investigation of a group of thirteen Californian soils from representative agricultural areas in the state. A comprehensive description of these soils as to classification, previous history as far as known, mechanical analyses, hygroscopic coefficients, and specific heats is given by Stewart (10) in a paper presenting the results of the studies of this group of soils during the first two years of the investigations. A more recent paper by Burd and Martin (1) describes the cultural conditions under which these soils have been maintained up to 1924 and reports certain observations on the liquid phases of the various soils, the pH of which range from 6.9 to 8.2.

Since the last mentioned observations were made, three additional crops of barley have been produced by the continuously cropped soils. The soils, which were uncropped from 1916 to 1925 inclusive, were cropped to barley in 1926, and are now under barley crops annually. The present study was undertaken to observe, if possible, changes which may have taken place in the replaceable bases of these soils. They have been maintained under very carefully controlled conditions in that nothing but distilled water has been added, nor has any leaching taken place during the entire period the soils have been under observation.

Concerning the effects of different treatments on the replaceable bases in soils, Page and Williams (6) at Rothamsted have observed those due to long continued annual fertilization of field plots. Smith (8) has noted in some Scottish soils the fairly permanent content of exchangeable bases for a given soil under normal conditions and also the variation from soil to soil. Steenkamp (9) has reported changes in the replaceable bases under the process of dehydration of soils. Of more direct bearing on the observations to be reported in this paper are those recently presented by Fraps (2). Using the ammonium chloride method in 88 tests of soils in pot experiments, he reports that the replaceable potash lost from the soil in supporting two crops is related to that contained in the crops with a correlation coefficient of 0.796.

Briefly, the treatments of these Californian soils since assembling at Berke-

[1] Laboratory of Plant Nutrition.

ley in 1915, are as follows: two duplicate galvanized iron containers (coated with black asphalt paint) were filled with each soil (1800 pounds); the first year all soils were cropped to Beldi barley; one container of each soil has been cultivated and cropped to barley annually up to the present time and in this paper is called the "cropped soil;" the other container of each soil was uncropped but cultivated annually and kept moist from 1916 to 1920, and from then till 1926 given no cultivation nor water; in 1926 the previously uncropped soils were again moistened and cropped to barley after which these observations were made. The soils which were uncropped for ten years but had supported a barley crop in 1915 and again in 1926 are called the "fallowed soils" in this paper. It is clearly recognized that the effect of fallowing alone is not measured in these observations. The term is used only for the sake of brevity in distinguishing them from the continuously cropped soils.

It is not the purpose of this paper to attempt to discuss the mechanism of base exchange, since there are numerous instances in the literature bearing on this phenomenon. It will be limited to a brief discussion of a set of observations made on this group of soils which have been under investigation for 13 years by our laboratory.

METHOD OF SAMPLING AND PREPARATION FOR REPLACEMENT

A portion of each soil was air-dried and screened through a 1-mm. screen in 1915 at the time the soils were assembled for experimentation. These portions have been stored in large stoppered bottles and in these observations are used as representative of the subsequently cropped and fallowed soils at the beginning of the experiment. The sampling of the cropped and fallowed soils was accomplished by taking ten uniformly spaced cores of soil from each soil, extending the entire depth of the container (18 inches). Each sample core was 1 inch in diameter. The ten cores of each soil were then composited, air-dried, and pulverized to pass the 1 mm. screen. The samples thus prepared were then used for the determination of replaeeable bases.

METHOD OF REPLACEMENT

The replacing reagent used was ammonium acetate adjusted to a pH of 7.0 and approximately normal, as suggested recently by Schollenberger (7). Twenty-five-gram portions of soil were each treated with about 150 cc. of the reagent in Erlenmeyer flasks, and allowed to digest at about 70°C. for an hour or two then transferred to a Buchner filter and the soil leached with reagent until the leachings showed no test for calcium. This usually required between 400 and 500 cc. of the reagent. The leachings were then made up to a volume of 500 cc. and determinations of the four major bases made in the usual way after removal of excess ammonium acetate, which is accomplished on the steam bath and finally on an air bath with moderate heating.

The results have not been corrected for water-soluble bases which these soils contain, as they are not significant in the magnitudes of the bases re-

ported. In a few cases, determinations have been made of the ammonium absorbed. In the heavy soils this value is about 80 per cent of the total determined bases extracted but in the light soils the agreement on the whole is not so close; in the case of soil 8 it is 55 per cent whereas in soil 12 it is 80 per cent of the bases extracted. Each value reported for the individual bases is the result of a single determination. The data are presented in table 1.

CLASSIFICATION AND TREATMENT OF SOILS

The first and second columns of the table describe the soil classification and give the laboratory numbers of the soils. It will be observed that there are five silty clay loam soils and one clay loam of the same series. The first three of these soils come from the Sacramento valley near Davis and the second three from the Santa Clara valley. It will also be observed that each fine sandy loam soil of this group is from a different series. Soil 7 came from near Arlington; soil 8 from the San Joaquin valley near Fresno; soil 9 from near Redlands; soil 10 from the San Fernando valley; soil 11 from Kearney Park in the San Joaquin valley; soil 12 from near Oakdale in the San Joaquin valley foothills; and soil 14, a virgin desert soil from eastern Lassen county. All of these soils are low in their $CaCO_3$ content, soils 2 and 9 being free from this compound while soils 4 and 5 contain the most, 0.20 and 0.18 per cent, respectively.

The third column shows the cultural treatment of the soils, and here it should be noted that the so-called fallow soils were cropped to barley in 1915 and again in 1926 at the end of which season these observations were made. During the intervening period of ten years these soils produced no crops.

REPLACEABLE BASES IN THE ORIGINAL SOILS

First, if we may confine our attention to the original soils, it will be observed that the amounts of replaceable bases are quite variable, ranging from 38.5 in soil 6 to as low as 6.8 milligram-equivalents in soil 8. As a group the heavy soils contain about twice the amounts present in the light soils. The clay and silty clay loam soils will be called "heavy" while the fine sandy loam soils will be called "light" for the sake of brevity. The spread of values in the former group (24.3 to 38.5 milligram-equivalents) although as great in actual amount as that of the latter group (6.8 to 20.0 milligram-equivalents) is relatively of smaller magnitude, as might be expected from the more variable nature and origin of the sandy loam soils. It will also be observed that there is more variability in the relative proportions of the four bases in the replaceable fractions of the light soils than is the case in the heavy soils. In the silty clay and clay loam group, the calcium and magnesium together comprise 94 to 96 per cent while the potassium and sodium are 4 to 6 per cent of the total. In the sandy loam group the calcium and magnesium together range from 83.7 to 92.2 per cent, potassium and sodium making up the remainder, 7.8 to 16.3 per cent of the total. It may be of interest to note that in soil 1 the magnesium

TABLE 1

Replaceable bases in original, cropped, and fallowed soils*

Description of soils			Per cent of water-free soil				Milligram-equivalents per 100 gm. of water-free soil		Relative proportion of bases (per cent of total)			
			Ca	Mg	Na	K	Total bases	NH_4 absorbed	Ca	Mg	Na	K
Yolo silty clay loam	1	Original	0.286	0.192	0.007	0.038	31.4	24.9	45.6	50.3	1.0	3.1
		Cropped	0.287	0.195	0.007	0.025	31.2	24.4	45.8	51.2	1.0	2.0
		Fallowed	0.282	0.194	0.012	0.033	31.5	24.6	44.7	51.0	1.6	2.7
	2	Original	0.322	0.101	0.008	0.053	26.0	20.1	61.6	31.9	1.3	5.2
		Cropped	0.322	0.090	0.007	0.041	24.9	20.1	64.6	30.0	1.2	4.2
		Fallowed	0.317	0.087	0.008	0.048	24.5	19.3	64.5	29.1	1.4	5.0
	3	Original	0.412	0.097	0.008	0.059	30.4		67.8	26.1	1.1	5.0
		Cropped	0.412	0.087	0.008	0.045	29.1		70.3	24.6	1.2	3.9
		Fallowed	0.415	0.090	0.007	0.050	29.7		69.7	25.0	1.0	4.3
	4	Original	0.345	0.074	0.006	0.031	24.3		70.8	25.0	1.0	3.2
		Cropped	0.345	0.074	0.004	0.022	24.0		71.7	25.3	0.7	2.3
		Fallowed	0.338	0.072	0.006	0.028	23.8		71.0	24.9	1.1	3.0
	5	Original	0.373	0.068	0.016	0.034	25.7		72.3	21.6	2.7	3.4
		Cropped	0.383	0.061	0.009	0.022	25.1		76.1	20.1	1.6	2.2
		Fallowed	0.376	0.067	0.011	0.031	25.6		73.4	21.6	1.9	3.1
Yolo clay loam	6	Original	0.453	0.171	0.023	0.032	38.5		58.7	36.5	2.7	2.1
		Cropped	0.453	0.157	0.026	0.021	37.2		60.7	34.7	3.1	1.5
		Fallowed	0.464	0.173	0.036	0.025	39.6		58.6	35.8	4.0	1.6
Mean of clay and silty clay loams		Original	0.365	0.117	0.011	0.041	29.4		62.0	32.8	1.6	3.6
		Cropped	0.367	0.111	0.010	0.029	28.7		64.0	31.9	1.4	2.7
		Fallowed	0.365	0.114	0.012	0.036	29.1		62.7	32.2	1.8	3.3
Hanford fine sandy loam	7	Original	0.191	0.031	0.011	0.022	13.1	9.0	72.7	19.3	3.7	4.3
		Cropped	0.194	0.032	0.013	0.016	13.3	8.8	72.8	19.8	4.3	3.1
		Fallowed	0.195	0.028	0.012	0.021	13.1	8.7	74.3	17.8	3.9	4.0
Fresno fine sandy loam	8	Original	0.090	0.017	0.009	0.021	6.8	3.5	66.0	20.4	5.6	8.0
		Cropped	0.093	0.018	0.008	0.007	6.6	3.7	70.3	22.2	5.0	2.5
		Fallowed	0.097	0.017	0.009	0.012	6.9	3.6	69.7	20.1	5.6	4.6
Kimball fine sandy loam	9	Original	0.118	0.021	0.007	0.014	8.2		71.6	20.6	3.5	4.3
		Cropped	0.129	0.020	0.007	0.010	8.6		74.5	19.2	3.3	3.0
		Fallowed	0.132	0.019	0.006	0.013	8.7		75.9	17.5	2.9	3.7
Tejunga fine sandy loam	10	Original	0.246	0.035	0.011	0.047	16.8		73.2	17.0	2.7	7.1
		Cropped	0.229	0.034	0.009	0.034	15.5		73.6	18.3	2.5	5.6
		Fallowed	0.238	0.034	0.010	0.042	16.2		73.5	17.3	2.7	6.5

* Soils cropped in 1915, fallowed from 1916–1925 inclusive, and cropped in 1926.

TABLE 1—*Continued*

DESCRIPTION OF SOILS			PER CENT OF WATER-FREE SOIL				MILLIGRAM-EQUIVALENTS PER 100 GM. OF WATER-FREE SOIL		RELATIVE PROPORTION OF BASES (PER CENT OF TOTAL)			
			Ca	Mg	Na	K	Totol bases	NH_4 absorbed	Ca	Mg	Na	K
Madera fine sandy loam	11	Original	0.182	0.049	0.010	0.045	14.7	10.7	61.8	27.4	3.0	7.8
		Cropped	0.187	0.046	0.008	0.029	14.3	10.4	65.6	26.7	2.5	5.2
Arnold fine sandy loam	12	Original	0.115	0.020	0.006	0.016	8.0	6.4	71.3	20.7	3.0	5.0
		Cropped	0.117	0.023	0.005	0.010	8.2	6.4	71.6	22.7	2.5	3.2
		Fallowed	0.112	0.023	0.004	0.015	8.0		69.9	23.4	2.0	4.7
Standish fine sandy loam	14	Original	0.255	0.049	0.011	0.108	20.0		63.5	20.2	2.5	13.8
		Cropped	0.258	0.048	0.013	0.091	19.6		65.2	20.0	2.8	12.0
		Fallowed	0.254	0.048	0.014	0.100	19.8		64.0	20.0	3.1	12.9
Mean of fine sandy loams		Original	0.171	0.032	0.009	0.039	12.5		68.6	20.8	3.4	7.2
		Cropped	0.172	0.032	0.009	0.028	12.3		70.5	21.3	3.3	4.9
		Fallowed	0.171	0.028	0.009	0.034	12.1		71.2	19.4	3.4	6.1

exceeds the calcium by about 5 per cent, the only case where this relationship exists. In the other heavy soils the calcium is from 1.6 to 3.3 times the magnesium equivalence. In soil 14 where we find the greatest amount of replaceable potassium, the ratio of this base to sodium is 5.5 to 1. Generally speaking, however, the amount of potassium is twice that of sodium. In comparing the mean value for the group of heavy soils with that of the group of light soils, it will be observed that calcium makes up a slightly greater proportion of the replaceable bases in the latter than it does in the former. The proportion of magnesium is considerably greater in the group of heavy soils. The proportions of potassium and of sodium in the group of light soils are both approximately twice the values for these bases in the heavy soils group, although the absolute quantities of each of these two constituents in the two groups are about equal.

It is of interest to note that an average of the relative proportions of the replaceable bases in these 13 soils gives values for calcium and magnesium which are quite close to those reported by Kelley and Brown (5) for an average of seven Californian soils of neutral or slightly alkaline reaction. In the latter, however, the sodium predominates over the potassium by a ratio of 2 to 1 while in the soils reported here the potassium exceeds the sodium by approximately the same ratio.

CHANGES IN THE REPLACEABLE BASES

In considering the data from the cropped and fallowed soils with respect to the condition of these soils at the beginning of the experiment, those changes

which are general and significant in magnitude over the entire group should receive major attention.

A comparison of the mean values for the cropped and fallowed soils of the heavy and of the light groups with those for the respective original soils, shows that in each group there has been only a slight and insignificant lowering of the values for total bases. The results for total bases for the individual soils, then, show that there are four cases each in which the cropped and the fallowed soils exceed slightly the original soils.

The mean values for each group of soils seem to indicate that calcium has remained constant, which is a reflection of the behavior of each heavy soil and of most of the light soils. In soils 8 and 9, both cropped and fallowed, there appear to be increases, but these are offset in calculating the mean values for the group by decreases in the case of soil 10.

As judged by the mean values for the group of heavy soils, magnesium shows a slight decrease in the cropped soils, the result of this behavior in four soils (2, 3, 5, and 6). In the cases of the fallowed heavy soils and both cropped and fallowed light soils, the magnesium seems to have remained practically constant.

Sodium seems to undergo no uniform or significant change, the mean values rather show it to be very constant, the result of about an equal number of increases and decreases in both groups of soils.

When we consider the mean values for potassium in each group there seem to be, especially in the cropped soils, very significant decreases. In most of the soils which had been fallowed, the decreases are not so large and in several cases cannot be considered significant. A comparison of the cropped and the fallowed condition of each soil with the corresponding original, shows that in every case there has been a loss. These decreases of replaceable potassium for the most part are very small in magnitude compared to the total replaceable bases but make up a very considerable part of the replaceable potassium present in the original soils. The potassium in the group of heavy soils comprises from 2 to 5 per cent of the total replaceable bases, with a mean value of 3.7 per cent, whereas in the light group it is from 4 to 14 per cent of the total, the mean being 7.2 per cent. It is evident, therefore, that significant changes in this constituent, which makes up such a small part of the replaceable fraction, might not alter the total of the replaceable bases by an amount which would be considered significant.

LOSSES OF REPLACEABLE POTASSIUM

Table 2 shows the losses of potassium, calculated first on the basis of the original content of this constituent, and second as percentage of the soil.

The values are very variable over the whole group of soils but it will be noted that the continuously cropped soils of the sandy loam type show a much wider spread of values both in absolute and relative losses. The behavior of the replaceable potassium in soil 7 is in striking contrast to that in soil 8. These

TABLE 2

Comparison of the losses of replaceable potassium from cropped and fallowed soils with that contained in the crops*

DESCRIPTION OF SOIL			LOSS FROM SOIL		CONTENT OF CROP (PER CENT OF WATER-FREE SOIL)
			Per cent of original	Per cent of water-free soil	
Yolo silty clay loam	1	Cropped	34.21	0.013	0.014
		Fallowed	13.16	0.005	0.007
	2	Cropped	22.64	0.012	0.014
		Fallowed	9.43	0.005	0.007
	3	Cropped	23.73	0.014	0.012
		Fallowed	15.25	0.009	0.006
	4	Cropped	29.03	0.009	0.014
		Fallowed	9.68	0.003	0.007
	5	Cropped	35.30	0.012	0.022
		Fallowed	8.82	0.003	0.009
Yolo clay loam	6	Cropped	34.37	0.011	0.013
		Fallowed	21.87	0.007	0.009
Mean of silty clay and clay loams		Cropped	29.88	0.012	0.015
		Fallowed	13.04	0.005	0.008
Hanford fine sandy loam	7	Cropped	27.27	0.006	0.012
		Fallowed	4.55	0.001	0.005
Fresno fine sandy loam	8	Cropped	66.67	0.014	0.016
		Fallowed	42.86	0.009	0.007
Kimball fine sandy loam	9	Cropped	28.57	0.004	0.008
		Fallowed	7.14	0.001	0.005
Tejunga fine sandy loam	10	Cropped	27.66	0.013	0.016
		Fallowed	10.64	0.005	0.008
Madera fine sandy loam	11	Cropped	35.56	0.016	0.016
		Fallowed	.....	.....	.....
Arnold fine sandy loam	12	Cropped	37.50	0.006	0.009
		Fallowed	6.25	0.001	0.005
Standish fine sandy loam	14	Cropped	15.74	0.017	0.012
		Fallowed	7.41	0.008	0.006
Mean of fine sandy loams		Cropped	34.14	0.011	0.013
		Fallowed	13.14	0.004	0.006

* Soils cropped in 1915, fallowed from 1916–1925, inclusive, and cropped in 1926.

two soils, starting with practically an identical quantity of this base, lost during the 12 years of cropping about 30 per cent and approximately 70 per cent, respectively, of the original supply. Soil 14 has an unusually high replaceable potassium content and this soil continuously cropped shows the greatest absolute loss, but it is also the smallest relative loss exhibited by any of the continuously cropped soils. It is interesting to note that in the cropped heavy soils, with the exception of number 4, an almost uniform amount of potassium was lost from each, and the relative losses are all within a comparatively narrow range, which might have been anticipated from the fact that each of these soils has been classified as belonging to the same series. It may be worth noting, however, that the mean value (0.012 per cent) for the loss of potassium from the group of cropped heavy soils is almost identical with the corresponding value (0.011 per cent) for the sandy loam group, although the relative loss from the former group is about 5 per cent lower than from the latter group. This may be a coincidence.

A literal interpretation of the figures indicates a loss of potassium from each of the fallowed soils. At least three of the soils (numbers 7, 9, and 12) cannot be said to have lost significant amounts. These are the same three soils which show the least absolute losses in the continuously cropped condition. Fallowed soils 4 and 5 show losses which may be considered barely significant, although in the continuously cropped condition their losses are certainly significant. The mean values for losses of potassium from the two groups (heavy and light) of fallowed soils are almost identical, 0.005 and 0.004 per cent, respectively. In the soils which were fallowed, we observe in the group of light soils a greater spread of losses (0.001 to 0.009 per cent) than in the group of heavy soils (0.003 to 0.009 per cent). The losses from fallowed soils 7 and 8 again show up in marked contrast, the former being about 5 per cent and the latter, 43 per cent, of their practically identical original contents.

These wide variations in magnitude of the losses of potassium seem to indicate that there are differences in the nature of the complexes involved in replacement. The differences appear to be accentuated in soils of variable origin, such as the sandy loam group.

COMPARISON OF LOSSES OF POTASSIUM WITH CROP WITHDRAWALS

The last two columns of table 2 show the loss of replaceable potassium from the soils under the two treatments and the amounts of potassium found in the crops removed from these soils, both expressed as percentage of the soil.

First, if we observe the mean values for each group, there are slightly greater amounts of potassium contained in the crops in each case than correspond to the observed decreases of this base in the soils. For the group of heavy soils in the continuously cropped condition, the loss of replaceable potassium accounts for 80 per cent of the potassium withdrawn by the crops from these soils whereas in the group of light soils the corresponding relation is 85 per cent. These are values which agree very closely with that reported by Fraps

(2) in his recent paper. When we compare the results for the individual continuously cropped soils, we find cases such as soils 1, 2, 3, and 6 of the heavy group, and soils 8, 10, and 11 of the light group where there are very close agreements between these values. On the other hand, we observe in the cases of soils 4 and 5 of the heavy group that the decreases correspond to approximately 60 per cent of the potassium found in the crops. In these last mentioned soils there appear to be no striking differences, either in their content of replaceable potassium or in its relation to the total, when compared to those heavy soils in which the losses agree closely with crop withdrawals. In the sandy loam soils 7, 9, and 12, where the decreases of replaceable potassium represent 50 to 70 per cent of the total amount of potassium removed by crops from these soils, we observe that this base constitutes an appreciably smaller proportion of the total replaceable bases than in those sandy loam soils where the decreases are closely related to crop withdrawals. The contents of replaceable potassium in the original soils 9 and 12 are considerably lower than any of the other soils either heavy or light, whereas that of the original soil 7 is the same as soil 8 before cropping; however, in the latter soil (no. 8) potassium constitutes a much greater proportion of the total replaceable bases.

The behavior of soil 14 is noteworthy in that the loss of potassium is significantly greater (0.005 per cent) than the crop withdrawal. It is the only continuously cropped soil in the entire group where this relation clearly exists. A characteristic of this soil distinguishing it from all the others is its unusually high content of replaceable potassium (0.108 per cent), which is approximately double the quantity contained in soil 3, the next in order of magnitude, and seven times the content of soil 9, which contains the least. Although it has been postulated by Page and Williams (6) that there may be a conversion of replaceable potassium to a non-replaceable form, the data here presented indicative of such a possibility are entirely too meagre to be convincing. Hissink (4) has stated that transfer between adsorbed and strong acid-soluble bases cannot occur to any great extent.

The bulk of the evidence at hand seems to indicate that the barley crops have either obtained part of their potassium from the non-replaceable form, or if it has all been absorbed from the replaceable complex, directly or indirectly, there has been a partial replenishment of the potassium in this complex from the non-replaceable form.

It will be observed that the losses of potassium from the so-called fallowed soils are more than accounted for by the two crops of barley removed from each of these soils. In the heavy group the loss is 63 per cent of the crop withdrawal, whereas in the light group it is 67 per cent, in both cases being somewhat lower than the corresponding relation in the respective continuously cropped groups. The striking observation again seems to be that the losses from the heavy soils 4 and 5 are one-half and one-third, respectively, of the crop withdrawals, whereas for the light soils 7, 9, and 12 the relation is one-fifth. To account for the behavior of the three last mentioned soils, one might venture the hypothe-

TABLE 3

*Twentieth-normal HCl extractable bases in original, cropped, and fallowed*soils*

Description of soils			Per cent of water-free soil: Ca	Mg	Na	K	Loss of K (per cent of water-free soil)
Yolo silty clay loam	1	Original	0.349	0.324	0.018	0.042	
		Cropped	0.365	0.325	0.018	0.026	0.016
		Fallowed	0.364	0.321	0.019	0.029	0.013
	2	Original	0.424	0.204	0.030	0.051	
		Cropped	0.431	0.218	0.017	0.056	0.005 (gain)
		Fallowed	0.428	0.215	0.025	0.055	0.004 (gain)
	3	Original	0.273	0.199	0.026	0.061	
		Cropped	0.275	0.203	0.025	0.045	0.016
		Fallowed	0.270	0.204	0.051	0.052	0.009
	4	Original	0.247	0.157	0.026	0.040	
		Cropped	0.253	0.167	0.037	0.026	0.014
		Fallowed	0.253	0.157	0.025	0.031	0.009
	5	Original	0.626	0.139	0.014	0.047	
		Cropped	0.642	0.144	0.013	0.028	0.019
		Fallowed	0.634	0.147	0.022	0.042	0.005
Yolo clay loam	6	Original	0.564	0.368	0.059	0.035	
		Cropped	0.584	0.374	0.049	0.024	0.011
		Fallowed	0.573	0.381	0.053	0.024	0.011
Mean of clay and silty clay loams		Original	0.414	0.232	0.029	0.046	
		Cropped	0.425	0.239	0.027	0.034	0.012
		Fallowed	0.420	0.238	0.033	0.039	0.007
Hanford fine sandy loam	7	Original	0.387	0.063	0.034	0.040	
		Cropped	0.357	0.061	0.025	0.030	0.010
		Fallowed	0.364	0.057	0.021	0.036	0.004
Fresno fine sandy loam	8	Original	0.210	0.035	0.008	0.029	
		Cropped	0.206	0.035	0.008	0.012	0.017
		Fallowed	0.222	0.031	0.012	0.021	0.008
Kimball fine sandy loam	9	Original	0.274	0.027	†	0.020	
		Cropped	0.301	0.027		0.020	0.000
		Fallowed	0.272	0.026		0.021	0.001 (gain)
Tejunga fine sandy loam	10	Original	0.567	0.044	†	0.047	
		Cropped	0.561	0.038		0.046	0.001
		Fallowed	0.557	0.042		0.044	0.003
Madera fine sand loam	11	Original	0.258	0.072	†	0.055	
		Cropped	0.265	0.073		0.039	0.016

* Soils cropped in 1915, fallowed from 1916–1925 inclusive, and cropped in 1926.

† Not determined.

TABLE 3—*Continued*

DESCRIPTION OF SOILS			PER CENT OF WATER-FREE SOIL				LOSS OF K (PER CENT OF WATER-FREE SOIL)
			Ca	Mg	Na	K	
Arnold fine sandy loam	12	Original	0.259	0.048	†	0.019	
		Cropped	0.269	0.049		0.019	0.000
		Fallowed	0.259	0.049		0.024	0.005 (gain)
Standish fine sandy loam	14	Original	0.357	0.124	†	0.126	
		Cropped	0.332	0.112		0.112	0.014
		Fallowed	0.355	0.115		0.124	0.002
Mean of fine sandy loams		Original	0.330	0.059		0.048	
		Cropped	0.327	0.056		0.040	0.008
		Fallowed	0.338	0.053		0.045	0.003

sis that the replaceable potassium had increased during the fallow period of 10 years. If this occurred, the losses of replaceable potassium would agree more closely with crop withdrawals. Unfortunately, the present observations were not made until the fallowed soils had been cropped for one season subsequent to the fallow period. It was observed, however, by Burd and Martin (1) that after eight years fallowing, the displaced solutions from soils 9 and 12 showed the greatest relative increases over their original concentrations; soil 7 remained practically constant, and soil 8 decreased to about half its original concentration. Soil 8, in the so-called fallow treatment, lost the greatest amount of replaceable potassium of any sandy loam soil, over 40 per cent of the amount originally present.

TWENTIETH-NORMAL HCl EXTRACTABLE BASES COMPARED TO THOSE REPLACEABLE BY AMMONIUM ACETATE

It has been suggested by Gedroiz (3) that a 0.05 *N* HCl extraction of a soil until the leachings are practically free from calcium gives a fairly accurate measure of the replaceable bases.

These soils were subjected to this extraction and the results of the analyses of the extracts are presented in table 3. (Compare with values in tables 1 and 2.)

The mean values for calcium in the group of heavy soils show that appreciably more of this constituent has been extracted by the acid. Two soils (no. 3 and no. 4) gave up more calcium to the ammonium acetate, while from one soil (no. 5) much more has been extracted by the acid. In the group of light soils the mean values show twice as much calcium extracted by the hydrochloric acid. Each soil in the latter group with the exception of number 14, shows approximately the same ratio (2:1) between the acid extractable and ammonium acetate replaceable calcium.

As regards magnesium, the entire group judged by the mean values for the heavy and for the light soils gave up twice as much to the 0.05 *N* HCl.

In each soil of the heavy group this ratio is practically constant. In numbers 9 and 10 of the group of light soils, the ratio is not very wide but is in favor of the acid, whereas from the other soils in this group the acid extracted twice as much magnesium.

The mean values for sodium in the group of heavy soils show that the ammonium acetate brought into solution only from 30 to 40 per cent of that extracted by the acid. In the two soils of the sandy loam type where sodium was determined, one (no. 8) shows a good agreement between the two extracting reagents. From the other (no. 7) the acid extracted approximately twice the amount of sodium.

The amount of potassium extracted by the hydrochloric acid is more closely related to that brought into solution by ammonium acetate than is the case with any of the other bases determined. The mean values for this constituent in the two groups of soils show the agreement of values to be a little better in the heavy soils. The ammonium acetate replaceable potassium ranges from 70 per cent, in the case of the group of cropped light soils, to 93 per cent, in the group of fallowed heavy soils, of that extracted by the 0.05 N acid.

The results of the extractions by either of these two reagents lead to the same conclusions with respect to calcium, magnesium, and sodium. The amounts of these three bases extracted by 0.05 N acid are appreciably greater than those brought into solution by ammonium acetate, but the cropping has not altered the values significantly.

When we consider the hydrochloric acid extractable potassium as affected by cropping, the results do not show losses over the entire group. There are cases such as soil 2 in which both replacing reagents give the same amount of this base before cropping but the acid extractable potassium seems to increase after crop growth. Again in soils 9 and 12 there are appreciable discrepancies between the amounts extracted by the two replacing reagents, especially after cropping. Soil 10 in the original condition and also in the so-called fallow treatment yields an equal amount of potassium to both reagents, but after continuous cropping shows no decrease of 0.05 N acid extractable potassium, as it did when treated with ammonium acetate. It is of interest to note that the losses of potassium from the cropped and fallowed soils number 4 and 5, as measured by the acid extraction, agree much more closely with those quantities withdrawn by the crops from these soils. There are other cases such as soil 3, before as well as after cropping, from which both these reagents extract the same amounts of potassium.

It might be stated that the quantities of silica, iron, and aluminum brought into solution by the 0.05 N HCl are of substantial magnitudes, which was not the case with the ammonium acetate extractions. This would indicate that the action of the acid was much more deep seated and was probably also bringing other silicates into solution.

To summarize the results presented in table 3 as compared to those in tables 1 and 2, then, the ammonium acetate treatment seems to be a closer

measure of the replaceable bases. The milligram equivalents of total bases brought into solution by this reagent are in much better agreement with the ammonium absorbed by the soils (where determined) than is the case with the 0.05 *N* acid extractable bases when calculated to this basis. The losses of potassium due to cropping are more consistent and quantitatively in better agreement with crop withdrawals of this base over the entire group of soils, when measured by the ammonium acetate replacement.

SUMMARY AND CONCLUSIONS

A survey of the data reported here leads to several conclusions:

There are greater relative variations in the amount of replaceable bases in soils classified as of different origin than within a group of soils of uniform classification. The quantities of exchangeable bases in these clay or silty clay loam soils are generally much higher than in the sandy loam soils.

When the soils were subjected to a prolonged period of cropping annually to barley or were cropped twice with a long fallow period intervening, there were no appreciable changes in the content of total replaceable bases.

There were significant decreases of replaceable potassium in all soils cropped annually for 12 years. Nine of the 12 soils, which supported two barley crops with a 10-year fallow period intervening, show significant decreases in potassium. The content of total bases is not significantly altered by these decreases in potassium, because calcium and magnesium, which comprise 90 per cent of the total, remain constant.

The loss of potassium from the entire group of soils supporting an annual crop of barley for 12 successive years is 32 per cent of that contained in these soils at the beginning of the experiment and is 82 per cent of the potassium removed in the crops from these soils. The losses of potassium from the same original group of soils, which have supported two crops of barley with an intervening fallow period of 10 years, are of questionable significance in several cases, but for the entire group, make up 13 per cent of the original content and constitute 64 per cent of the crop withdrawal.

The data seem to be especially significant, since this experiment has been conducted under conditions which preclude the loss of any of the four bases from the soil except by crop withdrawals.

These relations between losses of potassium and crop withdrawals of this constituent suggest the importance of the replaceable base complex with respect to available potassium in soils.

The 0.05 *N* HCl extractable bases in these soils are compared to the ammonium acetate replaceable bases and their relations discussed briefly.

REFERENCES

(1) Burd, J. S., and Martin, J. C. 1924 Secular and seasonal changes in the soil solution. *Soil Sci.* 18: 151–167.

(2) FRAPS, G. S. 1928 Relation of the replaceable potash of the soil to the potash removed by crops in pot experiments. (Paper presented before Div. of Agr. and Food Chem., Amer. Chem. Soc., April meeting, St. Louis.)

(3) GEDROIZ, K. K. 1923 The hydrochloric acid method for determining in the soil the cations present in an absorbed condition. *Soil Sci.* 16: 473–474.

(4) HISSINK, D. J. 1923 Method for estimating adsorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.

(5) KELLEY, W. P., AND BROWN, S. M. 1924 Replaceable bases in soils. Calif. Agr. Exp. Sta. Tech. Paper 15.

(6) PAGE, H. J., AND WILLIAMS, W. 1925 Studies on base exchange in Rothamsted soils. *Trans. Faraday Soc.* (No. 60) 20 (pt. 3): 573–585.

(7) SCHOLLENBERGER, C. J. 1927 Exchangeable hydrogen and soil reaction. *Science* 65 (1692 n. s.): 552–553.

(8) SMITH, A. M. 1928 Exchangeable bases in some Scottish soils. *Jour. Agr. Sci.* 18, (pt. 1): 68–75.

(9) STEENKAMP, J. L. 1928 The effect of dehydration of soils upon their colloid constituents: I. *Soil Sci.* 25: 163–182.

(10) STEWART, G. R. 1918 Effect of season and crop growth in modifying the soil extract. *Jour. Agr. Res.* 12: 311–368.

THE INFLUENCE OF ORGANIC MATTER AND LIME ON SOIL MOISTURE AND ON THE PERCENTAGE OF CARBON AND NITROGEN IN FIELD SOILS[1]

J. F. MULLER[2]

Rutgers University

Received for publication October 30, 1928

The field experiment on the availability of nitrogenous fertilizers at the New Jersey Agricultural Experiment Station has now been in progress for 20 years. Inasmuch as the treatments have been continuouly the same, with the exception of certain minor changes, the opportunity was taken of making a study of the moisture contents of the soils of certain of the plots as influenced by the fertilizer, manure, and lime treatments.

The experimental field is laid out into forty $\frac{1}{20}$-acre plots, one-half of which receive lime at the rate of 2 tons of ground limestone per acre every five years and one-half receive no lime. The unlimed plots comprise series A, the limed plots series B. The fertilizer treatments are the same for the corresponding plots in each series. Twelve plots, six in each series, were selected for this experiment, and the treatments which are given these plots are indicated in the following outline:

Plot number	*Fertilizer treatment*
4A, 4B	Minerals only*
5A, 5B	Minerals, 1600 pounds cow manure
7A, 7B	Nothing
18A, 18B	Minerals, 1600 pounds cow manure, 16 pounds $NaNO_3$
19A, 19B	Minerals only
20A, 20B	Minerals, 200 pounds wheat or rye straw, 16 pounds $NaNO_3$

* Minerals: 16 pounds acid phosphate, 8 pounds muriate of potash.

The soil is a Sassafras loam, which in some places contains considerable fine gravel.

The rotation for the first five years was corn, oats, oats, wheat, timothy; but in 1913 it was changed to corn, oats, wheat, timothy, timothy. The crop in 1928 was corn. The crop results have been reported in previous papers (1, 2, 3, 4).

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] The author, a graduate student at Rutgers University in the department of soil chemistry and bacteriology, is indebted to Prof. A. W. Blair for placing at his disposal the experimental plots, to Mr. A. L. Prince for the nitrogen and pH determinations, and to Prof. J. H. Clark for the rainfall records.

In this experiment nine sets of samples were taken, the first on May 1, the last on July 26. Eight borings, each to a depth of 6½ inches, were made for each sample. This composite sample was then quickly sifted through a 5-mm. sieve, well mixed and a portion of approximately one hundred grams placed in an aluminum weighing can. These samples were then weighed, dried to constant weight at 100°–105°C., and reweighed. The results are shown in table 1. The rainfall record covering the period of the experiment is shown in table 2.

The results in general are what might be expected in a soil of this type, namely, that the addition of organic matter increases the moisture holding capacity, and the periodic addition of lime, by coagulating the fine particles, decreases it. This is clearly shown in table 3, which gives the averages.

TABLE 1

Moisture contents of soils

PLOT	PER CENT MOISTURE									
	May 1	May 8	May 15	May 25	June 7	June 26	July 12	July 20	July 26	Average
4A	15.30	15.15	14.00	15.72	15.91	15.58	14.91	16.07	13.72	15.15
5A	14.56	14.94	14.26	15.53	15.60	15.38	14.94	14.14	12.74	14.68
7A	11.54	10.03	10.36	11.08	16.04	11.01	10.74	10.63	10.46	11.32
18A	15.49	15.71	14.84	15.78	16.38	16.10	15.13	13.80	12.71	15.10
19A	10.41	12.53	12.36	13.57	14.64	13.49	13.07	13.08	11.20	12.71
20A	14.35	14.18	13.11	14.37	15.77	13.25	13.28	12.12	10.44	13.43
4B	11.89	11.41	11.27	11.30	12.84	10.42	11.43	10.29	9.00	11.09
5B	13.23	13.09	13.58	13.71	15.59	14.01	14.09	13.74	11.50	13.62
7B	12.90	11.86	11.52	12.29	12.97	10.82	13.62	11.95	10.90	12.09
18B	14.97	15.62	15.71	16.04	17.54	15.84	17.13	17.36	12.40	16.29
19B	13.09	11.89	11.93	12.59	13.58	12.04	11.98	13.08	10.53	12.29
20B	14.15	13.00	11.95	12.73	13.56	12.75	12.36	11.49	11.88	12.65

It will be seen that the organic matter increases the moisture holding capacity more than twice as much as the lime decreases it. It is also shown that the effect of the organic matter is twice as great on the limed soils as on the unlimed. The differences between plots 4A and 5A were rather unexpected and are only explainable on the basis that 4A lies somewhat lower than 5A and thus receives some drainage water. In a season of as heavy rainfall as this, this tendency becomes more marked than would be the case in one of normal rainfall.

With the exception of the samples collected on July 20 and 26, the soils of plots 18A and 18B show the highest percentage of moisture in their respective series. The exceptions mentioned occur in connection with samples from plot 4A which show a higher percentage of moisture than the samples from 18A. The difference is no doubt due in part to the lower position of 4A and in

part to the fact that the corn on 18A was at this time making much more rapid growth and therefore was using much more moisture.

These soils were analyzed for total nitrogen and carbon; the results are given in table 4, together with the pH values. It is of particular interest to note the low percentage of carbon and nitrogen in the soil from plot 7A as contrasted with that from plot 5A, for example, which is separated from it by only about thirty-five feet. The percentage of nitrogen in 5A is practically double that in 7A. This change has been brought about in a period of 20 years. It is very evident that the manure has been an exceedingly important factor in maintaining the nitrogen and carbon content of the soil of plots 5A and 5B, and also of 18A and 18B.

TABLE 2

Rainfall record April 27 to July 24

DATE	MAY	JUNE	JULY	DATE	APRIL	MAY	JUNE	JULY
	in.	*in.*	*in.*		*in.*	*in.*	*in.*	*in.*
1	0.04	....	Tr.	16	....	....	....	....
2	....	0.11	0.05	17	....	Tr.	....	....
3	....	....	Tr.	18	....	0.67	Tr.	....
4	Tr.	0.27	0.72	19	....	0.13	0.73	....
5	....	0.76	1.18	20	....	0.05	0.15	0.12
6	....	1.38	1.96	21	....	Tr.	0.13	Tr.
7	....	Tr.	....	22	....	....	0.35	Tr.
8	....	....	....	23	....	....	Tr.	0.17
9	0.29	0.05	Tr.	24	....	....	0.33	0.06
10	0.05	0.94	0.07	25	....	....	....	....
11	Tr.	Tr.	0.02	26	....	0.23	Tr.	....
12	0.06	0.05	0.02	27	0.08	0.32	....	....
13	....	Tr.	2.13	28	1.11	0.02	....	....
14	....	Tr.	0.17	29	0.05	Tr.	0.57	....
15	....	0.98	....	30	Tr.	0.05	0.38	....
				31	....	....	....	....

The higher percentage of nitrogen and carbon in the soil from 7B as compared with 7A is undoubtedly the indirect result of the lime that is used on 7B. This plot has consistently given higher yields than 7A and this means larger crop residues returned to the soil; furthermore, on account of the lime, there has been a tendency for volunteer clover to appear on 7B after grain and hay have been harvested, and this has contributed some nitrogen and carbon.

Each year plots 18A and 18B give the heaviest crops for the respective series, and thus large crop residues remain to be plowed under for the succeeding crop. These residues, together with the manure that is applied, add large quantities of organic matter, which is reflected in the increased moisture content and a higher percentage of nitrogen and carbon in the soil of these plots.

Analyses made in 1909, soon after the laying out of the experimental plots, showed the percentage of total nitrogen and carbon in these soils to be 0.112 and 1.22 respectively (1). Table 4 shows that the content of total nitrogen in the soils of 18A and 18B is now 0.134 per cent. This is an increase of 440

TABLE 3

Average moisture content of soils

TREATMENTS	MOISTURE
	per cent
Average of all receiving organic matter	14.30
Average of all receiving no organic matter	12.44
Increase due to organic matter	1.86
Average of all receiving lime	13.01
Average of all receiving no lime	13.73
Decrease due to lime	0.72
Average—unlimed with organic matter	14.40
Average—unlimed without organic matter	13.06
Average—limed with organic matter	14.19
Average—limed without organic matter	11.82

TABLE 4

Percentage of carbon and nitrogen and pH values

PLOT NUMBER	TOTAL NITOGEN	TOTAL CARBON	pH VALUES
	per cent	*per cent*	
4A	0.100	1.16	5.36
5A	0.135	1.51	5.46
7A	0.068	0.71	4.70
18A	0.134	1.79	5.88
19A	0.084	1.15	5.03
20A	0.098	1.32	5.80
4B	0.078	1.04	7.05
5B	0.132	1.75	6.87
7B	0.077	1.09	7.20
18B	0.134	1.73	7.09
19B	0.081	1.14	7.04
20B	0.092	1.24	7.29

pounds of nitrogen per acre 6⅔ inches in 20 years, or 22 pounds per acre per year. Over the same period, 7A shows a loss of 880 pounds of nitrogen, or 44 pounds per acre per year. Plots 20A and 20B, which receive the same amount of nitrate of soda annually as 18A and 18B, show losses of 14 and 20 pounds of nitrogen per acre per year respectively, so that it is apparent that the increased

nitrogen content of the soils of 18A and 18B is due mainly to the large quantity of organic matter which they receive.

The six soils receiving annual applications of organic matter, either as straw or manure; namely 5, 18, and 20, A and B, show an increase in total carbon over that originally present in 1909, whereas the six which have had no added organic matter all show a decrease in total carbon.

SUMMARY

1. A series of moisture determinations was made on the soils of certain experimental plots.
2. In all cases but one, those soils receiving an annual application of organic matter show a higher moisture content than those receiving no treatment or only minerals.
3. In all cases but two, those soils receiving lime show a lower moisture content than the corresponding soils receiving no lime.
4. The addition of organic matter increased the moisture content approximately twice as much as the addition of lime decreased it.
5. The soils receiving annual applications of organic matter, as manure or straw, show an increase in total carbon over the amount originally present in 1909. Those receiving manure show an increase in total nitrogen also. All other plots show a decrease in these two elements.

REFERENCES

(1) Lipman, J. G., and Blair, A. W. 1913 Field experiments on the availability of nitrogenous fertilizers. N. J. Agr. Exp. Sta. Bul. 260.
(2) Lipman, J. G., and Blair, A. W. 1920 Field experiments on the availabilty of nitrogenous fertilizers, 1908–1917. *Soil Sci.* 9: 371–392.
(3) Lipman, J. G., Blair, A. W., and Prince, A. L. 1925 Field experiments on the availability of nitrogenous fertilizers, 1918–1922. *Soil Sci.* 19: 57–59.
(4) Lipman, J. G., Blair, A. W., and Prince, A. L. 1928 Field experiments on the availability of nitrogenous fertilizers, 1923–1927. *Soil Sci.*, 24: 1–21.

LIME PENETRATION RESULTING FROM SURFACE APPLICATION TO PASTURE LAND[1]

P. R. NELSON

Received for publication November 24, 1928

Considerable conflicting data have been obtained by different investigators on the penetration of lime into different depths of the soil. Wilson (10) found no downward movement of calcium in pots after 12 months of weathering. Practically no penetration was obtained in a sod-covered lysimeter after one year at the Pennsylvania Agricultural Experiment Station (4). Ruprecht (5) showed that an application of lime was almost completely removed from the soil in a lysimeter after a 10-year drainage water study. In a crop rotation study Stewart and Wyatt (7) found in one field, after 3½ years, that there was no evidence of penetration; but in a second field, limed for 14 years, there was a partial neutralization of the second 6 inches. White and Holben (9), as a result of 40 years continuous application of fertilizer to the soil, state that of the 13 per cent of lime lost from the surface, 65 per cent was retained in the subsoil. Van Alstine (8), studying surface applications of lime to Rothamsted soils, found a slight penetration of calcium. McIntire (3) reports that lime applied in a 4-year rotation for 30 years is more completely disseminated through the first 21 inches on a manured soil than on an unmanured soil.

EXPERIMENTAL

The present paper gives the results of a study of the penetration of lime into a Gloucester sandy loam in permanent pasture plots having received different fertilizer treatment. The field consisted of six series of eleven plots, 2 square rods in area, each of which was divided into an east and west half, with ground limestone applied to the east portion in the fall of 1921 at the rate of 2400 pounds per acre. Eighteen of the plots received no fertilizer and were used as a check on the influence of the fertilizer. Those plots to which the greatest application of fertilizers had been made were studied, it being felt that the possibility of an influence upon the lime penetration would be more apt to be manifested in them. In the fall of 1927, samples were taken by Prof. C. P. Jones and the author at depths of 0 to 3, 3 to 6, 6 to 9, and 9 to 12 inches from the eighteen check plots and from those which received an average total application of 640 pounds of potassium chloride, 3840 pounds of acid phosphate, or 1920 pounds of gypsum per acre during the period of the experiment. The

[1] From the department of chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the director of the station.

TABLE 1

Analyses of the pasture soils

DEPTH	KCl				ACID PHOSPHATE				GYPSUM				CHECK			
	Limed		Unlimed		Limed		Unlimed		Limed		Unlimed		Limed		Unlimed	
	CaO	pH	CaO	pH	CaO	pH	CaO	pH	CaO	pH	CaO	pH	CaO	pH	CaO	pH
inches	*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
0–3	0.580	5.70	0.440	4.82	0.623	5.68	0.447	4.99	0.628	5.96	0.454	5.10	0.667	5.70	0.452	4.83
3–6	0.425	5.02	0.420	4.81	0.405	4.95	0.438	4.82	0.453	5.19	0.426	4.90	0.486	4.99	0.454	4.75
6–9	0.458	4.82	0.474	4.82	0.403	4.94	0.424	4.85	0.451	5.10	0.442	4.95	0.488	4.93	0.439	4.85
9–12	0.460	4.85	0.458	4.86	0.427	5.02	0.425	4.92	0.417	5.04	0.420	4.95	0.506	4.99	0.483	4.94

samples were air-dried, and soil particles larger than one millimeter in diameter were discarded.

Two methods for observing the effect of the lime were used: determination of calcium oxide by Hilgard's method, and hydrogen-ion by means of the quinhydrone electrode (8).

From the results shown in table 1, the amount of calcium on the surface of the limed areas indicates that the greater part of it has remained at the point of application, since it is nearly 0.2 per cent greater than on the unlimed portions and also greater than in the lower zones of the limed areas. At lower depths in all cases, except the limed check plots, where there seems to be a trend toward accumulation, there is little evidence of penetration. Indeed, in the case of the acid phosphate and gypsum plots, where considerable more calcium has been added, more of the residual lime has been leached from the soil.

The pH determinations, on the other hand, give indication of penetration in each type of fertilization, which is not apparent from the calcium determinations. With the exception of the gypsum plots and the surface layer of the acid phosphate plot, the unlimed plots show no change in soil acidity as a result of fertilization. The gypsum has shown a tendency, though slight considering the amount which has been added, to neutralize the acidity to a depth of 9 inches. This is not in agreement with most of the results obtained with gypsum. Erdman (2) reports with an application of 1000–2000 pounds per acre that there is an increase in acidity of .21–.27 pH. Cubbon (1), with several soil types, is in agreement with the majority of the workers that gypsum has no effect on soil acidity. The large increase in pH of the surface soil on the limed portions of the plots corresponds favorably to the increase in calcium found in these same plots, but in addition, there are significant differences at lower depths which were not manifest by the calcium determinations. The check plots exhibit a slight penetration as shown by a partial neutralization of the soil acidity to the depth of 9 inches. Acid phosphate apparently has no influence upon the pH, for the results agree closely with those of the check plots at the same depths; but potassium chloride seems to have a tendency to retard the penetration, for neutralization has not occurred beyond the second zone. If the neutralizing effect of the gypsum alone is removed, the same manifestation of penetration is obtained in these plots as is obtained with lime alone or with acid phosphate and lime.

SUMMARY

From a study of the lime content and the pH values of the treated and untreated pasture soils it has been found that the greater part of the calcium has remained at the point of application and exerted its influence there. The pH values show a trend toward a gradual penetration as indicated by a slight, though consistent, neutralization of the soil acidity to a depth of 9 inches. The

only fertilizer which has had the slightest neutralizing effect upon the soil is gypsum and its effect has not influenced the change in acidity caused by the application of lime alone.

REFERENCES

(1) Cubbon, M. 1925 Calcium sulfate as a soil amendment. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 97.

(2) Erdman, L. W. 1922 The effect of gypsum on soil reaction. *Soil Sci.* 12: 433–448.

(3) McIntire, W. H. 1913 Results of thirty years of liming. *Penna. Agr. Exp. Sta. Ann. Rpt.* 1911–12: 64–75.

(4) Pennsylvania Agricultural Experiment Station. 1922 Lime top-dressing experiment. *Penna Agr. Exp. Sta. Bul.* 170: 24.

(5) Ruprecht, R. W. 1921 Report of physiological chemist. *Fla. Agr. Exp. Sta. Ann. Rpt.* 1920–21: 23R.

(6) Snyder, E. F. 1927 A comparative study of the quinhydrone and hydrogen electrodes for determining the hydrogen-ion concentration of soils. *Jour. Agr. Res.* 35: 825–834.

(7) Stewart, R., and Wyatt, F. A. 1919 Limestone action on acid soils. *Ill. Agr. Exp. Sta. Bul.* 212: 265–296.

(8) Van Alstine, E. 1918 The movement of plant-food within the soil. *Soil Sci.* 6: 281–308.

(9) White, J. W., and Holben, F. J. 1926 Residual effects of forty years continuous manurial treatment: III. Ultimate fate and some physical chemical effects of applied lime. *Soil Sci.* 22: 61–74.

(10) Wilson, B. D. 1918 Translocation of calcium in soil. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 17: 289–320.

AN IMPROVED SOIL-SAMPLING TUBE

F. J. VEIHMEYER

California Agricultural Experiment Station

Received for publication November 21, 1928

Experience extending over a number of years with different types of tools designed to sample the soil for the determination of its moisture content has shown that a soil-sampling tube properly made gives more accurate and consistent results than those obtained with other devices. In fact, under certain conditions it has been found that the soil tube is the only device which can be used. Where gravel is encountered the pieces may be large enough to prevent the use of either the "post-hole" or "worm" augers, but with the sampling tube described herein it is possible to cut through the gravel and obtain a satisfactory sample. The disadvantages of the soil-sampling tube are its high cost and the difficulty sometimes encountered in removing the tube from the hole in dry soils.

The sampling tube which has been found best suited where quantitative measurements of soil moisture are desired is similar to the tubes usually referred to as the "King soil tube." However, certain changes in the sampling tubes heretofore designed have been found to better to a considerable degree the performance of these devices.

The details of the sampling tube, together with the drive head and point (fig. 1), are sufficiently clear for any competent machinist to follow. Tubes over 5½ feet long are not convenient to use unless holes are first punched with shorter tubes. The maximum length of tubes in one piece used by the writer is about 13 feet. This tube can be used for taking samples to a depth of 12 feet, but three other tubes, a 4½-foot, a 7-foot, and a 10-foot tube, are first used. These three tubes take the samples from 0 to 3 feet, 3 to 6 feet, and 6 to 9 feet, respectively. The tubing is made of seamless cold drawn steel containing 3.5 per cent of nickel.

The success of the sampling is largely determined by the shape of the drive point. The point shown in figure 1 was adopted after trials were made with many other points of different shapes. The point is shaped so that a core of soil is cut without pushing the soil ahead of the point and compacting it, or without compacting the core in the tube. This fact is clearly evidenced by the satisfactory results of measurements of the apparent specific gravity, or volume weights, of soils in place obtained with this tube. The soil tube used by Beckett[1] to make volume-weight determinations was the tube described herein.

[1] Beckett, S. H. 1928 The use of highly viscous fluids in the determination of volume-weight of soils. *Soil Sci.* 25: 481–483.

Beckett's results show that this tube may be used with confidence for this purpose.

If the point is properly tempered many samples can be taken even in gravelly soil before the point is worn out. However, when either points or heads need replacement or repair they may be removed readily by unscrewing them from the tubes. This also is a unique feature since all other tubes, as far as the writer is aware, have the heads and points permanently attached to the tubes. The taper in the point from $\frac{7}{8}$ inch at the outer end to $\frac{31}{32}$ inch at the inner end has been found to be an essential feature. Points designed by others have straight holes about up to the open end and the opening is sharply constricted. This arrangement is supposed to prevent the core

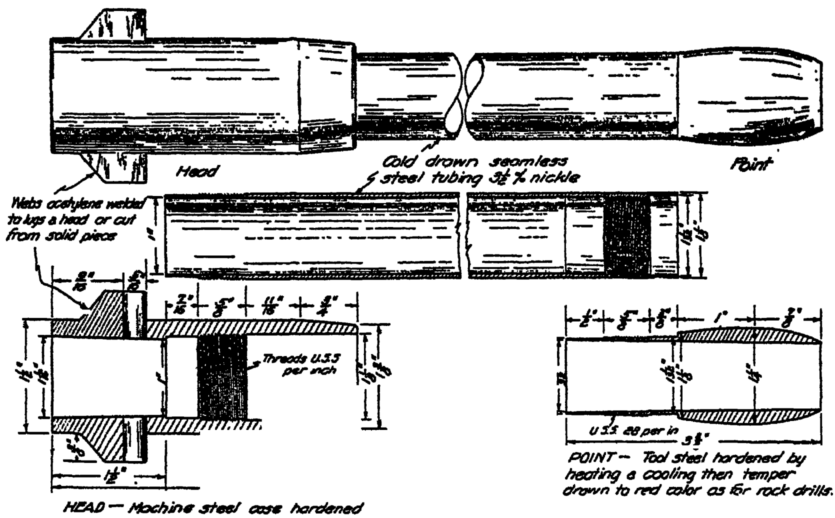


Fig. 1. Details of Soil-sampling Tube

of soil from being pulled out of the tube when the tube is withdrawn from the hole. The use of a straight bore in the drive point is objectionable since it tends to compact the core and offers resistance to the entrance of the core into the tube. The loss of the core can be prevented with the point of the present design by lightly compacting the soil in the tube with a pointed rod before pulling the tube out of the hole. Samples were taken with this tube in fine sand, so dry that other devices could not be used, by pushing the soil lightly with a rod which caused the samples to stay in the tube on withdrawal from the hole. In saturated or nearly saturated clays, samples can not always be kept in the tube but the insertion of a rubber cork in the open end of the head may keep the suction, which results from removing the tube from the hole, from pulling the core out of the tube. The use of the rubber cork in

addition to tamping the core is advisable in all cases where extreme care is desired to retain all of the core in the tube, for instance, as when making volume-weight determinations.

The shape of the head is not so important as that of the point but experience has shown that the head must be tapered on the inside as shown in figure 1. In the present design this taper is from 1 inch at the shoulder to $1\frac{1}{16}$ inches at the outer end of the head. A straight hole in this portion of the head causes the core of soil to lodge and it can be removed only with difficulty. The lugs on the head may either be made as shown in the sketch or the head may be turned down from a large piece of steel, the space between the lugs being planed off. Either arrangement is satisfactory and the cost is about the same

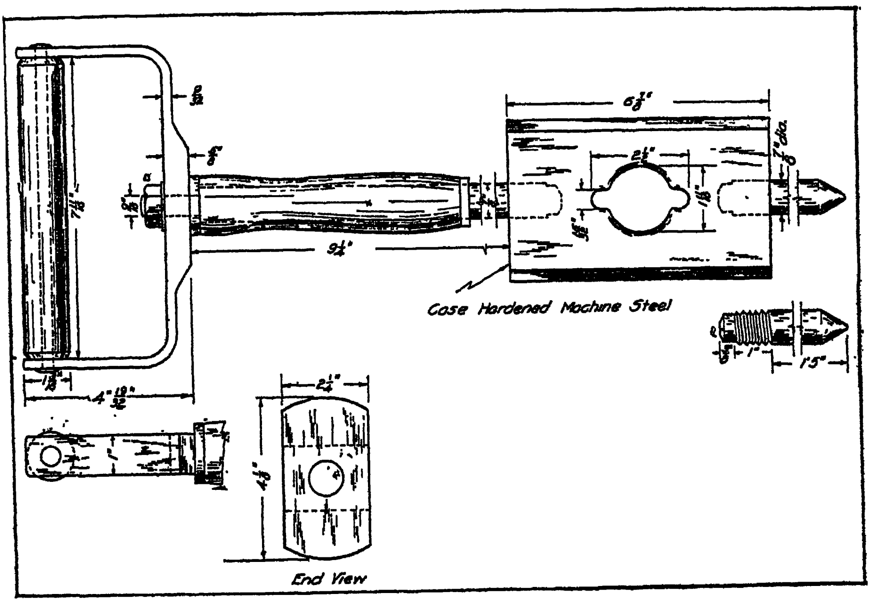


FIG. 2. HAMMER TO DRIVE SOIL TUBE

with either method of construction. When made in large quantities the heads may be cast or drop-forged.

While the hammer may be made in a number of different ways, the one shown in figure 2 has proved very convenient. The hammer shown weighs about 18 pounds. A lighter hammer is not satisfactory except in moist soils. In sampling dry soils a heavier hammer, one which is $7\frac{1}{4}$ inches long, $4\frac{11}{16}$ inches wide, and $2\frac{1}{4}$ inches thick, weighing about 30 pounds, has been found more desirable. The hammer may be made also of cast steel which is subsequently hardened by chilling. When several orders for manufacturing hammers are placed with one machinist the cost may be lessened considerably, since the patterns for the casting can be used a number of times.

The drive hammer can be used to remove the tube from the hole. Usually in moist soils it is sufficient to pass the hammer over the head and give it a quarter turn and pull the tube by means of the lugs. When the tube sticks in dry soils, it may be knocked out of the hole by bringing the hammer up against the lugs with a sharp blow. The core of soil in the tube must be compacted before an attempt is made to withdraw the tube in this way, otherwise it will

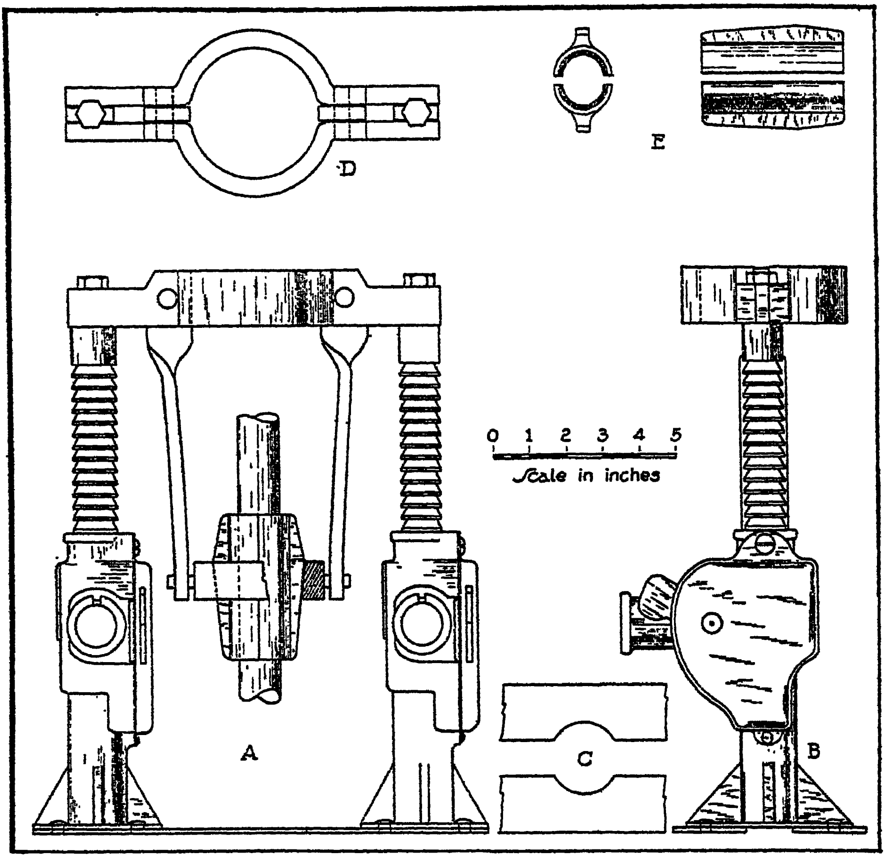


FIG. 3. PULLER FOR SOIL TUBE

be jarred out of the tube The tapered portion of the inner end of the head facilitates withdrawing the tube by jarring it with the hammer. It should be noted that the head and hammer are hardened to prevent them from being battered out of shape. Hardening of the head and hammer is further necessitated by the fact that the core can be removed from the tube satisfactorily only by jarring the head on the hammer. It is never desirable to attempt to push the core out of the tube.

Under some conditions soils are encountered in which the expediency just described of hammering the tube may not be sufficient to pull the tube from the hole and it is necessary, therefore, to use a puller. Figure 3 illustrates a puller which has never failed to work successfully, although its bulk and slowness of operation are disadvantages.

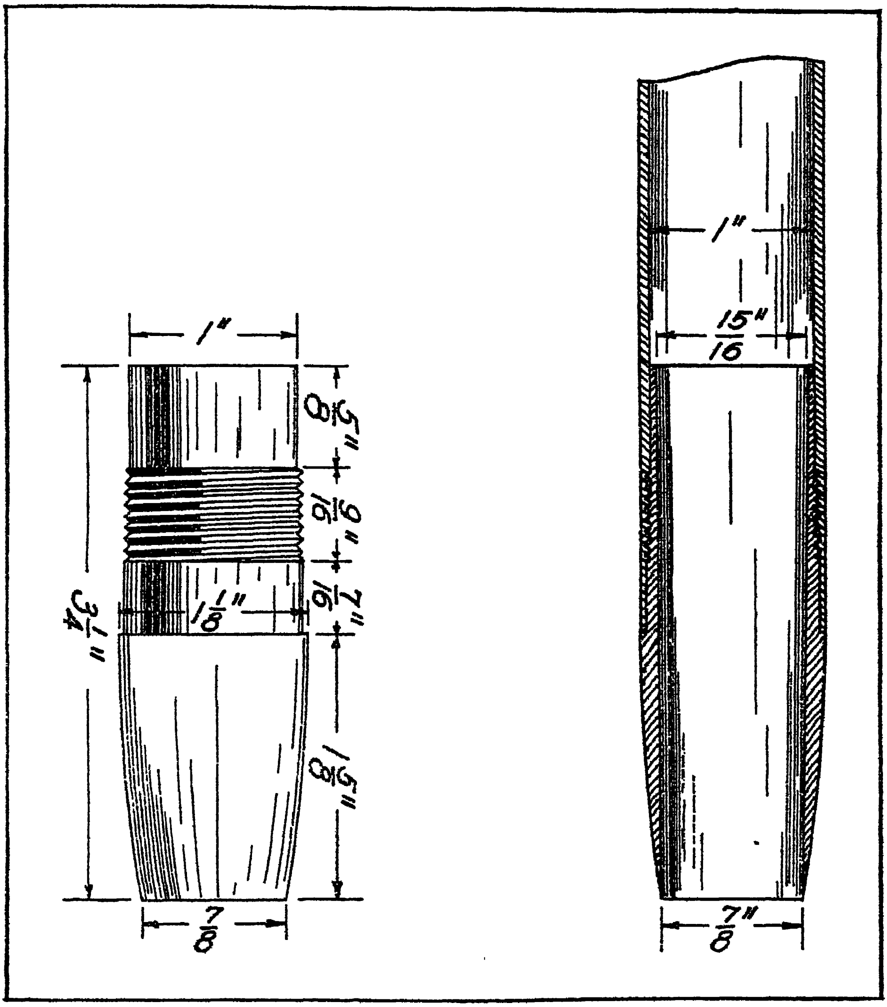


FIG. 4. SOIL TUBE POINT FOR USE IN DRY SAND

The puller consists of two automobile jacks mounted on a base and connected at the top by means of a yoke. The yoke is provided with a circular central opening (fig. 3 D) and the soil tube can be passed through this opening and an opening in the base (fig. 3 C). A ring is suspended from the yoke by

means of two links. The inner surface of the ring is beveled as shown in figure 3 A. Two jaws which are lined with fibre fit into the ring. The diameter of the inner surface of the jaws is the same as the outer diameter of the sampling tube. The rib on each jaw (fig. 3 A, E) is beveled the same as the inner surface of the ring. The operation of pulling the tube from the hole with this device is as follows: The puller is placed over the tube which passes through the opening in the base, ring, and yoke. The jacks are lowered until they occupy their lowest position. They are then raised by working in unison the handles (not shown in the figure). The sliding of the inner surface of the ring on the ribs of the jaws causes the jaws to move together and to grip the tube tightly. An increase of the pull exerted on the tube increases the force with which the jaws grip the tube; the fibre lining of the jaws increases the friction between the jaws and the tube. The inner surfaces of the jaws, having the same diameter as the tube, do not crush the tube even though tremendous forces are developed in pulling the tube. The jacks shown in figure 3 have been found to be most satisfactory since after being raised to their upper limit they will drop back to their lowest position if the trip (fig. 3 B) is released and the handles of the jacks are raised at the same time.

The difficulties, under certain conditions, of removing the tube from the hole have always been a pertinent objection to soil-sampling tubes. The tube with the point described herein offers less resistance to withdrawal from the hole than any of the many kinds which were tested in different soils, and the writer believes its proportions should not be altered except in cases where extreme types of soil are to be sampled. Where deep samples are desired the performance of the tube can be bettered by slightly decreasing the maximum diameter of the point (given as $1\frac{1}{4}$ inches in figure 1) for each tube, the tube with the smallest diameter being used in the deepest hole. In fine sand, a point shown in detail in figure 4 has given good results in taking samples below the upper three feet. The maximum diameter of the point is the same as the outer diameter of the tube so that there is no clearance. Consequently, the sand which is shaken from the sides of the hole when the tube is driven deeper does not lodge behind the point as is the case when points with clearance are used. The point shown in figure 4 can not be used easily in moist loam or clay soils since the soil grips the tube throughout its length and great effort is required to pull the tube from the hole.

Successful operation of the tube depends upon its being kept clean. A small flue cleaner ground down to fit loosely into the tube and screwed onto the end of a rod, when wrapped with steel wool and used in the same manner as a shot gun cleaner, has proved to be very satisfactory for this purpose. The tube is always wiped out with an oil-soaked cloth after being used. When it becomes necessary to remove either the head or the point the tube should never be clamped in a vise nor held with a wrench in such a manner as to dent the tube, because denting prevents the core from being removed easily from the tube. The jaws of the puller (fig. 3 E) can be placed around the tube and clamped in a vise to hold the tube when the head or point is being unscrewed.

EFFECTS OF CARBON DISULFIDE TREATMENT OF SOIL FOR THE JAPANESE BEETLE ON THE ABUNDANCE OF MICROORGANISMS AND ON THE AMMONIA AND NITRATE CONTENT[1]

WALTER E. FLEMING

Bureau of Entomology, U. S. Department of Agriculture[2]

Received for publication November 26, 1928

During the past six years several hundred thousand nursery and greenhouse plants have been grown successfully under commercial conditions in soils which have been treated with carbon disulfide according to the recommendations of the Japanese Beetle Laboratory to destroy the immature stages of the Japanese beetle. Carbon disulfide is used as a gas (1) and also as an emulsion (6) to exterminate the eggs and larvae of the beetle in the soil. Nurserymen have found that many varieties of greenhouse plants usually grow as well in this fumigated soil as in unfumigated soil under normal commercial conditions. The growth of ornamentals which are treated in the field by pouring a dilute emulsion on the soil about their roots is usually inhibited for a short period after treatment, after which they are stimulated into vigorous development.

In view of the great economic value of the ornamental and greenhouse crops that are treated annually, it was decided that further information regarding the effect of these insecticidal treatments on soils should be obtained by studying the results on the numbers of microörganisms and on the accumulation of ammonia and nitrates in the soil.

Many different types of soil are used to grow the various nursery and greenhouse plants. Because of the limited time available for the work, it has been possible to make this preliminary microbiological study on Sassafras loamy sand only. The results obtained, however, are probably indicative of those which would be obtained with other soils. The preliminary results are presented at this time, because there is little possibility of conducting in the near future an extensive investigation of the microbiological effects of the treatments.

It is realized that, under certain conditions, there may be no relationship between the number of microörganisms and the accumulation of their products of metabolism and the growth of higher plants, since other factors than the supply of nitrogeneous matter and the decomposition of plant residues may influence development. In general, it seems reasonable to expect

[1] Contribution No. 47, Japanese Beetle Laboratory, Moorestown, N. J.

[2] The writer is indebted to Dr. J. G. Lipman, director, and to Dr. S. A. Waksman, soil microbiologist, of the New Jersey Agricultural Experiment Station, for suggestions and for the use of equipment during the course of this work.

that plants will not grow properly in a normal, fertile soil if the insecticidal treatment has seriously reduced the number of microörganisms and has caused the accumulation of relatively large quantities of ammonia. If, on the other hand, the treatment has stimulated the development of microörganisms, and has tended to speed up the nitrifying processes, it is possible that the treatment will have been beneficial to the plants.

METHODS OF DETERMINING THE NUMBERS OF MICROÖRGANISMS AND THE QUANTITIES OF AMMONIA AND NITRATE IN THE SOIL

The method used in counting the number of bacteria in the soil is that given by Waksman and Fred (10). A suspension of soil in sterile water is diluted to a convenient and known extent, depending upon the number of bacteria present. One cubic centimeter of the diluted suspension is placed upon a sterile Petri dish and is mixed with melted nutrient agar medium. In these experiments five plates which were prepared for each soil were incubated at 25° to 28°C. for seven days. The number of bacteria per gram of soil was then determined by counting the number of colonies which developed on the plates. The number of fungi in the soil was determined by a similar method, the special acid medium recommended by Waksman (9) being used, and the incubating period being three days. The nitrates were determined colorimetrically with phenol disulfonic acid, and the ammonia was distilled from the soil into a standard sulfuric acid solution in the presence of magnesium oxide, and was determined by titration. Both these methods are described by Fred (4).

EFFECT OF CARBON DISULFIDE VAPOR

A quantity of Sassafras loamy sand was sifted to remove stones and large lumps of soil and to make it homogeneous. It was then divided into 2-kgm. samples, which were placed in jars. One cubic centimeter of carbon disulfide was added to each of five jars, which gave a concentration equivalent to that produced by a dosage of 1 pound per cubic yard. Five untreated jars were used as a control, and all were sealed for a period of 48 hours. The covers were then removed, and sterile cotton plugs were inserted to permit the vapor to escape without subjecting the soil to possible contamination by the bacteria and fungi of the air. No trace of carbon disulfide could be detected in the jars at the end of five days, when the solid covers were replaced. The samples were kept at room temperature, and sterile distilled water was added to each as required to replace that lost by evaporation. Variation in the number and activity of the microörganisms present in the different samples was prevented, to a large extent, by maintaining the samples at optimum moisture content and at a fairly constant temperature.

The number of bacteria and fungi and the quantity of ammonia and nitrate in each sample were determined before the soils were treated with the carbon disulfide. Determinations were also made after 7 days, when the odor of the chemical had disappeared from the soil, and after 33, 66 and 112 days.

The results of these determinations, which are given in table 1, indicate that carbon disulfide applied at the rate of 1 pound to a cubic yard and left in contact with the soil for 48 hours has little effect on the number of bacteria. The treatment stimulated the development of fungi in the soil, the density being approximately three-fold that of the untreated soil throughout the 112 days of observation. There was no change in the concentration of nitrates but there was an accumulation of ammonia in the treated soil.

TABLE 1

Effect of carbon disulfide vapor on soil

(Carbon disulfide used at the rate of 1 pound per cubic yard of soil)

DAYS AFTER BEGINNING OF EXPERIMENT	FUMIGATED SOIL				UNTREATED SOIL			
	Number of organisms		Activity of organisms		Number of organisms		Activity of organisms	
	Millions of bacteria per gm.	Thousands of fungi per gm.	Mgm. of ammonia per 100 gm.	Mgm. of nitrate per 100 gm.	Millions of bacteria per gm.	Thousands of fungi per gm.	Mgm. of ammonia per 100 gm.	Mgm. of nitrate per 100 gm.
0	4.1	16.8	0.60	0.146	3.8	19.7	0.60	0.150
7	5.8	71.0	0.69	0.150	5.1	22.6	0.65	0.146
33	5.3	86.1	1.00	0.146	6.2	29.1	0.60	0.146
66	4.5	95.0	1.10	0.155	5.8	31.0	0.70	0.150
112	3.1	104.3	2.00	0.150	3.1	36.0	0.90	0.150

TABLE 2

Effect of carbon disulfide emulsion

DAYS AFTER START OF EXPERIMENT	SOIL TREATED WITH CS_2 EMULSION				SOIL TREATED WITH WATER				UNTREATED SOIL			
	Number of organisms		Activity of organisms		Number of organisms		Activity of organisms		Number of organisms		Activity of organisms	
	Millions of bacteria per gm.	Thousands of fungi per gm.	Mgm. of ammonia per 100 gm.	Mgm. of nitrate per 100 gm.	Millions of bacteria per gm.	Thousands of fungi per gm.	Mgm. of ammonia per 100 gm.	Mgm. of nitrate per 100 gm.	Millions of bacteria per gm.	Thousands of fungi per gm.	Mgm. of ammonia per 100 gm.	Mgm. of nitrate per 100 gm.
0	3.0	27.0	0.20	0.146	2.9	28.0	0.20	0.146	3.0	27.6	0.20	0.146
3	0.2	1.0	0.00	0.146	2.5	3.0	0.00	0.146	2.9	27.8	0.00	0.146
10	21.0	2.4	1.00	0.146	2.9	4.6	0.00	0.146	3.8	28.0	0.20	0.146
17	30.0	3.1	0.90	0.121	5.0	12.9	0.60	0.186	4.0	27.0	0.30	0.146
23	16.3	4.0	0.89	0.126	7.7	29.3	0.60	0.200	5.1	25.2	0.00	0.150
30	17.6	8.4	2.15	0.150	4.0	37.6	0.60	0.186	6.2	29.1	0.00	0.146
59	16.4	32.9	2.00	0.150	3.7	32.0	0.60	0.150	5.0	28.0	0.20	0.150
150	5.6	66.6	2.10	0.150	3.5	28.0	0.80	0.150	3.1	36.0	0.60	0.150

EFFECT OF CARBON DISULFIDE EMULSION

Six 4-kgm. samples of sifted Sassafras loamy sand were placed in inverted bell jars, the lower openings of which had been covered with muslin and were

treated by percolating 4 liters of a dilute emulsion containing 2 gm. of carbon disulfide through each mass. An equal number of samples was treated in the same manner, but with water containing no carbon disulfide, and a third group, consisting of untreated samples, was placed in glazed earthen jars to serve as controls. The treatment of loose soil with large quantities of water or of dilute emulsion altered the structure of the soil and caused it to puddle. As the samples were still saturated with water after three days, they were removed from the bell jars and spread on heavy paper to dry. After two weeks, when the moisture content of the treated soils was about 50 per cent saturation, the samples were placed in glazed earthen jars and kept at 50 per cent saturation by the addition of sterile distilled water as required. Samples of each soil were taken at intervals for determination of the number of bacteria and of fungi and the quantity of ammonia and nitrate present.

The results of these determinations, shown in table 2, indicate that the treatment of loose Sassafras loamy sand with a large quantity of water causes a decrease, followed by an increase, in the number of bacteria and fungi in the soil. The increase in the number of organisms was accompanied by an increase in the rate of accumulation of ammonia.

When diulte carbon disulfide emulsion was used in place of water, there was a more marked decrease in the number of bacteria and fungi, followed by a greater increase in the number of these microörganisms. There was also a greater accumulation of ammonia, and a slight decrease in the proportion of nitrates.

RESULTS

The fumigation of Sassafras loamy sand with carbon disulfide at the rate of 1 pound to 1 cubic yard caused a change in the number of bacteria and of fungi per gram of soil and in the concentration of ammonia. On the seventh day after treatment, when all odor of the fumigant had disappeared, the number of bacteria per gram was almost the same as in the original soil, and this did not change greatly throughout the 112 days of observation. In view of the facts established by Waksman and Starkey (11), Hiltner and Stoermer (5), Russell and Hutchinson (7, 8), Fred (2, 3), and others, it is probable that a slight reduction in the concentration of bacteria in the soil would have been observed if a determination had been made while the fumigant was present in the soil. It is also possible that if more than 1 pound of the carbon disulfide had been used, a more pronounced reduction of the number of bacteria, followed by a rapid increase in their density, would have been observed, such as normally occurs when soil is partially sterilized with higher concentrations of this compound. The fungi, on the other hand, were greatly stimulated by fumigation with the small quantity of carbon disulfide that was used. The fungous flora, which was simplified by the treatment, consisted largely of species of *Zygorhynchus* and of *Penicillium*. These fungi were three times as abundant in the treated soil as all fungi in the original soil. The nitrate content of the

soil was not appreciably reduced; the ammonia increased from 0.6 mgm. to 2 mgm. per 100 gm. of soil. In view of these changes, it is probable that the organic matter in the soil had been modified so as to be more available to higher plants. The accumulation of ammonia in the treated soil might prove detrimental to the growth of some greenhouse plants, particularly if the soil were stored where it was not well aerated and if the nitrifying organisms could not develop readily. It appears to be important, therefore, that, in treating soil by this procedure, precautions be taken to aerate the soil thoroughly after fumigation to prevent the accumulation of ammonia in toxic concentrations.

Treatment of loose Sassafras loamy sand with 0.05 per cent carbon disulfide emulsion resulted in puddling of the soil and a great change both in the numbers and in the nature of the microörganisms present. Fungi were almost eliminated, and recovered very slowly. Bacteria were destroyed, but became active and multiplied rapidly as the excess moisture evaporated, so that by the end of the seventeenth day they were ten times as numerous as in the original soil. There were few nitrifying organisms left in the soil, however, because the ammonia content had increased from 0.2 mgm. to over 2 mgm. per 100 gm. It remained at this high concentration for 120 days, while the nitrate content was appreciably reduced. It is apparent that a loose soil, when treated with a large volume of dilute emulsion, such as was used in this test, suffers a detrimental change in its physical structure. The decrease in nitrates and the accumulation of ammonia are probably injurious to higher plants, and may account, in part, for the injury sustained when the soil remains puddled or saturated with dilute emulsion for several days. In well-drained, undisturbed soil in the field the effect of treatment with carbon disulfide emulsion probably resembles that of fumigation, since thousands of nursery trees have been treated successfully on soils of this type.

SUMMARY

A Sassafras loamy sand was treated with the concentrations of gaseous and of emulsified carbon disulfide used for the immature stages of the Japanese beetle, and observations were made on the effects on the microörganisms and on the concentration of ammonia and nitrates in the soil.

Treatment with 0.05 per cent carbon disulfide (1 pound to 1 cubic yard) did not affect the density of the bacterial population appreciably, but it stimulated the development of fungi. It also caused an accumulation of ammonia.

Treatment under laboratory conditions with 0.05 per cent carbon disulfide emulsion was not satisfactory. The detrimental effect on the bacteria and fungi, the accumulation of ammonia, and the decrease in the concentration of nitrates in the soil are probably factors involved in causing the injury following treatment of ornamentals in poorly drained fields, or in fields puddled by the application of large volumes of water. It is probable that the results of treatment of well-drained, fertile soils with this concentration of carbon disul-

fide emulsion are very similar to those of fumigation when the same quantity of the gas is used.

Treatment with 0.05 per cent carbon disulfide, either as gas or as emulsion, results in an accumulation of ammonia and in a change in the numbers of microörganisms. Care should be taken to aerate the soil after fumigation, and the emulsion treatment should be used only in well-drained soil, so as to avoid, as far as possible, excessive accumulations of ammonia.

REFERENCES

(1) Fleming, W. E. 1923 Fumigation of potting soil with carbon disulfide for the control of the Japanese beetle. *N. J. Dept. Agr. Bul.* 380: 1–45.

(2) Fred, E. B. 1911 Ueber die Beschleunigung der Lebenstaetigkeit hoeherer und niederer Pflanzen durch kleine Giftmengen. *Centbl. Bakt.* (2) 31: 185–245.

(3) Fred, E. B. 1916 Relation of carbon bisulfide to soil organisms and plant growth. *Jour. Agr. Res.* 6: 1–19.

(4) Fred, E. B. 1916 A Laboratory Manual of Soil Bacteriology. Philadelphia.

(5) Hiltner, L., and, Stoermer, K. 1903 Studie ueber die Bakterienflora des Ackerbodens, mit besonderer Beruecksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. *Arb. Biol. Abt. Land. Forstwirtschaft Kais. Gesundh. Amt., Abt.* 3: 445–545.

(6) Leach, B. R., Fleming, W. E., and Lipp, J. W. 1927 Control of Japanese beetle grubs. *Penna. Dept. Agr. Bul.* 440: 15–21.

(7) Russell, E. J., and Hutchinson, H. B. 1909 The effect of partial sterilization of soil on the production of plant food. *Jour. Agr. Sci.* 3: 111–144.

(8) Russell, E. J., and Hutchinson, H. B. 1913 The effect of partial sterilization on the production of plant food. II. The limitation of bacterial numbers in normal soil and its consequences. *Jour. Agr. Sci.* 5: 152–221.

(9) Waksman, S. A. 1922 A method for counting the numbers of fungi in the soil. *Jour. Bact.* 7: 339–341.

(10) Waksman, S. A., and Fred, E. B. 1922 A tentative outline of the plate method for determining the number of microörganisms in the soil. *Soil Sci.* 14: 27–28.

(11) Waksman, S. A., and Starkey, R. L. 1923 Partial Sterilization of soil, microbiological activities, and soil fertility. *Soil Sci.* 14: 137–157, 247–268, 343–357.

AMERICAN SOILS AS SEEN BY RUSSIAN INVESTIGATORS

J. S. JOFFE

New Jersey Agricultural Experiment Station

AND

I. ANTIPOV-KARATAEV

Dokuchaev Soil Museum, Leningrad

Received for publication December 10, 1928

The First International Soil Science Congress held in Washington, D. C., in June, 1927, and the transcontinental tour through the United States and Canada of over two hundred delegates opened a new chapter in the development of soil science. For the first time in the history of this relatively young science a gathering of the most prominent representatives from all over the world has come about. Almost all the branches of soil science have been brought together and an inventory of the accumulated knowledge has been made. These have been correlated and systematized, and a great program for the furtherance of the science has been laid down. The discussions at the meetings and the interchange of views have had a profound effect on the immediate methods of attack of various soil science problems and have opened new avenues of approach to the many complex problems of the science.

One of the outstanding features of the Congress was the "invasion" of the Russian viewpoint on soils. The large, illustrious delegation with the late Dr. K. D. Glinka as the leader made a concerted effort to put across the Dokuchaev school of soil science, a purely Russian creation. An attempt was made by the Russians to fit into their scheme the soils of the North American continent. Their plan is based on comparative morphological soil characters and the climatological and geological data placed at their disposal by the American workers in the field of soil science. Much was drawn from the extensive store of knowledge of American soils possessed by Dr. Marbut, who prepared an elaborate guide for the members of the soil tour.

Several of the Russian investigators who participated in the meetings and in the tour have since published their observations. The purpose of this paper is to summarize their views. Of the five papers that came to the attention of the reviewers, two—one by Kravkov and the other by Tulaikov—are of a general nature and deal but little with the soil phase of the Congress and tour. On the other hand, the papers by Prasolov (1), Vilenskii (3), and Tyurin (2). are concerned with the soil science aspect of their observations and findings.

I. Vilenskii presents a sketchy account of the general geographical characters of the United States and southern Canada. He deals with the orography,

geology, climate, and, somewhat in detail, the flora of the country. With this as a basis, Vilenskii constructs a classification and makes up a map of the soil cover of the country presenting two soil maps: one of the soil regions as submitted by Marbut and one of his own.

Vilenskii fits the soils of the United States and Canada into a system of zonal distribution of soils: 1. The soils of the subtropics (thermophytogenic[1]); 2. The soils of the temperate belt (phytogenic); 3. the soils of the cold belt (phytohydrogenic). Besides these zonal belts one may find intrazonal: 1. Solonetz-like soils (phytohalogenic); 2. Solonchak (hydrohalogenic). In addition to the intrazonal belts Vilenskii gives also the extrazonal-marsh soils (hydrogenic)—and the vertical zones—mountain soils (orogenic).

All of the mentioned types of soil were found by Vilenskii in the United States and Canada. Still he recognizes that "*in America there is not the zonation of soils in our sense of the word, neither latitudinal nor longitudinal, but there is a checkerboard pattern in which each soil square will to a certain degree be similar to the one either to the south or west. This, however, is only true for the soils of the central plain; the soils from the southeastern portion of the United States are distributed in belts running from northwest to southeast.*" This peculiar distribution is explained by Vilenskii[2] as follows: "In North America the direction of the isohyetal line is in general perpendicular to the direction of the isothermal, while in Russia they are more or less parallel."

In describing the various soils of the United States and Canada, Vilenskii uses the comparative method, always comparing the types of soil encountered with the known types of Russian soils. Thus he describes the soils in Minnesota (near St. Paul) and Illinois (near Moline) as podzolized soils on a red, carbonate-free moraine. The whitish podzolized horizon terminates at a depth of 30 to 40 cm. The podozolized soils were also found in the Canadian Rockies at Jasper Park on various parent materials such as limestone and local moraines. Podzolized soils were also encountered in the Appalachian region, from the southern portion of Indiana and Ohio. But there the soils "may belong to the yellow-red division of soil formations, being an intermediary formation between the true yellow-red and the primary podzolized soils of the Great Lake region. . . . . "

"Grey forest soils were found in many places: in Minnesota, Alberta (near Edmonton), Iowa, and the southern portion of Missouri, where these soils are developed on loess. .. . . ."

"The chernozem type of soils was found in the prairie soils of Canada and at Fargo, North Dakota. Some degraded chernozem was encountered in Iowa and Missouri. The southern type of chernozem was found in the form of a gray variation in central Kansas at Fort Hays. . . . ."

[1] The nomenclature in parenthesis is original with Prof. Vilenskii. In general, however, the system of zonal distribution of soils dates back to Dokuchaev and Sibirtzev. J. S. Joffe.

[2] The reason for this peculiar distribution expounded by Vilenskii was explained by Dr. Marbut on several occasions while on the Soil Science Congress tour. J. S. Joffe.

"Typical chestnut soils[3] were not met, as we crossed that region by train at night."

Vilenskii points out that the majority of their delegates saw for the first time the subrropical soils. "There is no ground, as Glinka pointed out, to subdivide the yellow and red soils as distinct types of soil formation, as they are stages of the soil formation in one and the same subtropical zone. The yellow soil is an early stage of subtropical weathering and the red soil developing parallel with the yellow is a later stage. The representative types of this soil were found in Virginia, North Carolina, Georgia, and Tennessee. They are found even further north, in New Jersey and Pennsylvania. . . . ."

"Because of the development of the yellow and red soils on several parent materials and because of variation in relief, these soils are very different in their color and other properties."

The reddish brown soils of California are a puzzle to Vilenskii. He says: "One may argue whether the red coloration is due to the soil-forming processes or whether it is a property of the soil-forming parent material." And again: "Thus the red type of soil formation and the red soils of America are distributed under conditions of abundant precipitation in a continental climate of the eastern states, in the desert climate of the high plateau of the Rockies, and on the Pacific coast, irrespective, in the latter case, of the rainfall. It is therefore necessary to depart from the view that these soils are a product of 'the moist tropical and subtropical climate.'"

Finally Vilenskii touches upon the characteristic features of the salinized soils of America. He agrees in a way with Widtsoe (Yearbook of the U. S. Department of Agriculture, 1921) that "the great deposits of alkali and alkali-impregnated soils are associated with the geological history of the country. In early geological days salt lakes, similar to the Great Salt Lake, were no doubt formed which were dried by the changing climate leaving great masses of salt that were later covered by washings from the hills."

Vilenskii, who has studied alkali soils quite extensively, promises to treat the alkali soils of America in a separate paper.

II. Prasolov[4] speaks highly of the work of the Bureau of Soils, especially the division of soil survey and mapping. He finds fault with the maps, which are difficult to read because of the poor selection of colors and the inconsistency of nomenclature of soils in the various sections of the country. "The maps are in part a sort of an agricultural land survey. They represent an arbitrary classification of soils based on the separation of 'types' according to their mechanical composition, and 'series of types' according to the sum of their properties, but in the limits of a given region. At present there are 240 series and 2900 types. In recent years Dr. Marbut and his associates have con-

[3] These follow the southern type of chernozem in the Russian scheme of soil classification. J. S. Joffe.

[4] The section on Prasolov's observations has been written up entirely by Antipov-Karataev and translated by Joffe.

structed a new system of classification based not on the sampling of accidental layers of soil, but on the study of the natural cuts and their genetic horizons."

Prasolov presents the proposed system of Marbut and compares it with the known Russian classification. The new system is as follows:

1. Podzol group in the northwestern states.
2. Jersey group of yellow and brown soils (the "brown earth" of Ramann).
3. Red-brown soils of the south.
4. Dark soils of the prairie in the central states (corresponding chiefly to the leached chernozem of Russia).
5. The Dakota group-steppe soils (corresponding to the Russian southern chernozem).
6. The group of the dry western steppe soils (corresponding to the chestnut soils found in southern Russia).
7. Desert soils (corresponding to the grey soils of Turkestan).

The author carefully presents the facts which justify the classification. First he explains the cause of the meridinal distribution of soil zones in America. It is the meridinal distribution of the mountains in the western part of North America and the absence of high mountains on the Atlantic coast that are responsible for the phenomenon observed. For this reason the eastern states represent temperate-humid and forest regions. As we go westward these regions change gradually first into the prairie region with a more pronounced continental type of a climate and then into the steppe and desert regions. The break takes place at the Rocky Mountains. The end of the desert region is located in the depression between the Rockies and the Sierras. The disturbance in the proper zonation is brought about by the influence of the warm gulf stream from the Gulf of Mexico, which runs alongside the southeastern coast of North America. The continentality of the southern climate is thereby obliterated. On the other hand, the cooling influence of the cold Laborador stream and of the Hudson Bay on the northwestern portion of the country is worth mentioning. These two reasons change the picture of the soil cover in the eastern portion of North America, placing the podzolized soils in the northern forest region, the "brown soils of Ramann," somewhat to the south, and the red soils in the south. The same forces influence the prairie soils.

Prasolov discusses the prairie soils quite extensively. In the neighborhood of Kansas City he found degraded chernozem, and 400 kilometers west of it, the so-called southern chernozem. The same type of soil was found in the southern portion of Canada. From there it extends south to the parallel 310 north latitude in Texas. The author is reluctant to draw too close an analogy between the prairie soils and the Russian chernozem, considering that "the analogy to the prairie chernozem may perhaps be found in the region of the southern mountain steppe."

After enumerating several other local series of chernozem soils[5] as given by

[5] Prasolov describes the chernozem soils in Iowa, which take in about ten series with an acid reaction; the chernozem of Canada, which formed on a carbonate moraine; the chernozem which developed from rendzina; and the extreme members of the chernozems—the humid-meadow-humus soils with a carbonate horizon, near Winnipeg.

the American scheme of classification, and describing their characteristic features, Prasolov stresses the importance of the problem of comparing the Russian and American classification.

"West of the prairie the plain rises, the climate becomes more dry, salinizing processes appear, and the zone of chestnut soils is formed with the well-known flora of our southeast." Prasolov goes on to describe the light chestnut, somewhat salinized soils with the characteristic columnar structure in the horizon at a depth of 20–50 cm. "Further west, in the southern portion of California the grey soils may be noted; one may find there alkali soils with a lower compact marl layer."

Prasolov makes a point in noting the soils in the more humid belt of California where the Mediterranean type of climate prevails. There one may find the surface layers of the soil to be light brown and leached. Whenever these soils "formed either on the eluvium of the parent rock or on the ancient transported deposits, the lower horizons attain a reddish tinge." Similar soils may be found on the southern shore of Crimea.

III. The paper of Tyurin on the soils of North America is based not only on observations of morphological characters of the soils, but also on some chemical analyses made on samples procured by the author. It is therefore a fitting supplement to the papers of Vilenskii and Prasolov.

According to Tyurin the territory of the United States and the southern portion of Canada may be divided into three chief regions distributed in a meridianal direction. The climatic conditions, character of vegetation, and prevailing types of soil formation may serve as a basis for these broad divisions.

"The eastern region represents a forest area, characterized by a humid climate with soils that belong to the podzol type of soil formation. It includes the Coastal Plain, the Appalachians, and the adjacent region toward the west and northwest portion of the Mississippi basin. . . . ."

"Next comes the plains region extending to the Rockies. This has been a grass country until settlements were established. The grass cover is richer in the eastern, more humid region (tall grass), than in the dry western region (short grass). This is the region of chernozem-like, chernozem, and chestnut soils which distribute themselves in a meridianal direction and which alternate as we go westward. . . . ."

"The last region is that of the Rocky Mountains, characterized by a dry climate and by desert or semi-desert vegetation and soils."

Tyurin, like his colleagues, recognizes "that because of the peculiar relations between temperature and rainfall in North America with respect to longitude and latitude, the soil-forming processes are influenced and each region may be subdivided into a series of belts not only in meridinal but also in latitudinal direction."

With the tentative classification of Dr. Marbut (see review of Prasolov's paper) as a basis, Tyurin presents data first on the New Jersey group. The eastern representatives of this group possess the characteristic features of the

podzolized type of soil formation. It differs from the true podzolized soils by its clearly expressed brownish or yellowish-brown coloration of the B horizon. Tyurin considers the western representatives of this group as genuine podozolized soils. These soils developed under mixed forests at an average yearly temperature of 12°C. and 1200 mm. of rainfall.

Tyurin made the following determinations: hygroscopic moisture, humus, loss on ignition, chemically combined water, absorbed bases, and pH.

The Sassafras series (N-E representative of the New Jersey group) take a position intermediate between the true podzolized soils and the red soils. They approach the red soils, inasmuch as they have an accumulation of the sesquioxides in the B horizon, as judged by the high amount of absorbed CaO (0.24 per cent) and MgO (0.02 per cent). The humus content drops from 7.32 per cent in the A_1 horizon to 0.27 per cent in the B horizon.

The western representatives of soils do not differ from the podzolized soils in their humus content (5.84 per cent in A_1 and 0.45 per cent in B), chemically combined water (1.38 per cent in A_1, 1.42 per cent in A_2, and 3.36 per cent in B), and absorbed bases (0.041 per cent CaO and 0.014 per cent MgO in A_2; 0.4 per cent CaO and 0.17 per cent MgO in B). These analyses are on the Miami series in Indiana.

The southern representatives of this group present a peculiar problem. In a way, they are yellow soils as judged from the color of the A horizon, while they may be looked upon as red soils as judged by their B horizon. Tyurin concludes that it may become necessary to discard the term "yellow soil," as it seems to be an intrazonal formation in the zone of the red soils, agreeing to call "red soils" those which have a red B horizon.

The amalgamation of the yellow and red soils into one subgroup is in agreement with the scheme of Dr. Marbut for these soils.

These soils, having the structural features of the podzolized soils, must be classified with them: the A_1 horizon is shallow, 3–5 cm. deep with a slightly dark coloration of humus; A_2 is 15–20 cm. deep with a light-yellowish coloration, which gradually changes into an orange-red in the B horizon. These soils differ from the typical podzolized soils in that they have a low content of absorbed bases (0.006 per cent CaO and 0.003 per cent MgO in A; 0,01 per cent CaO and 0.003 per cent MgO in B), a low capacity for base exchange, and an unsaturated condition. The high content of chemically combined water in the B horizon (6.88 per cent) seems to be an indication of the destruction of the constituents to the point of the hydrates of the sesquioxides and to a decrease in the degree of dispersion.

The region of the plains is divided by Tyurin into three belts: 1. Prairie plain, 2. Chernozem, 3. High plain-chestnust soil.

The author thinks that the prairie belt "reminds one of the forest steppe in the boundaries of the degraded chernozem." Alongside the degraded chernozem type of soils the author found in this belt also some brown soil (Knox soil). Tyurin is inclined to place the Cherokee soils into a type which devel-

oped after having gone through some solonetz stages. The presence of amorphous silica seems to substantiate his findings. Tyurin does not commit himself about these soils, since his considerations are based on one analysis only.

The northern portion of this belt contains more humus (7.88 per cent in the Clarion soils) than does the southern portion (3.05 per cent humus in the Marshal soil). Both soils are slightly acid and in both of them horizon B has an accumulation of the colloidal fraction which came down from the A horizon. The amount of absorbed bases and the chemically combined water in these soils bring them close to the degraded chernozem.

The soils of this belt developed under conditions of grass vegetation with a moisture regime capable of supporting forests, which, however, were not apparent in this belt because of prairie fires, as pointed out by Shantz.

The next belt—the chernozem—occupies a territory of 150–200 km. and traverses the states, beginning in central Texas, through Oklahoma, Kansas, Nebraska, western portion of South Dakota, North Dakota, Manitoba, Saskatchewan, and Alberta. It stretches over a distance of 2000 km. from north to south. The temperature and rainfall in the northern portion being different from those in the southern, make the representative types of the soils also differ. As a representative of the southern group, Tyurin takes the soil at Fort Hays, Kansas, with 3.41 per cent of humus, 0.50 per cent absorbed CaO, and 0.066 per cent MgO in the A liorizon. The northern group as represented by the soils from Brandon, Manitoba, Saskatoon, and Edmonton have a higher humus content in the A horizon, namely 7.39 per cent, 7.38 per cent, and 16.03 per cent respectively.

The third belt extends from the second up to the Rockies with still less precipitation and about the same temperature as in the chernozem belt. Analyses are presented of the soil from Tribue, Kansas. This soil, notwithstanding its lighter color, contains about the same amount of humus—3.15 per cent—as the soil at Fort Hays, which belongs to the chernozem belt. At the Rockies the soils begin to resemble the type of brown soils.

Tyurin considers the soils of the Rocky Mountains as soils of a typical mountain region with characteristic vertical zonation, and speaks but little of these soils. Not much is said about the soils west of the Rockies and no experimental data are presented.

Tyurin sums up his report as follows:

1. Because of the specific physico-geographical conditions of the North American continent the chief soil zones, which may be characterized by definite types of soil formation and which coincide with the chief zones of natural vegetation, are distributed in a longitudinal direction. This zonality of the first order is primarily the result of a corresponding distribution of atmospheric precipitation on the territory of the continent.

2. Within the borders of the zones of the first order, because of temperature differences from north to south, certain subzones in respect to the latitude may be noted; these may be considered as zones of the second order. They are differentiated by various types of soil formation.

3. In addition to the chief natural factors in soil formation—temperature and rainfall,

which determine these zones—there are local and sometimes more than local factors of soil formation which influence the soil in the same direction as temperature and rainfall. At times these factors influence the soil formation in the reverse direction thereby causing certain departures from the zonations indicated. Of these factors the most important are: the age of the soil, drainage conditions, the parent material, and the human factor. Examples of the significance of the first factors were noted on the red soil group, also on the northern portion of the chernozem belt. The presence of a special subzone, caused by the human factor, may be found in the chernozem-like soil of the eastern portion of the prairie.

4. Summing up all the factors of soil formation and their territorial distribution, the North American continent presents many peculiarities as one attempts to compare it with the portion of Eurasia known to us. A more complete study of these peculiarities will offer a chance to develop that viewpoint in soil science which has as a basis the idea of geographicity of soils.

The review of the papers by the three Russian investigators shows a marked similarity—with the exception of minor differences on the intrazonal soils—of opinion, which is based on the system of zonal divisions of soils. This fact seems to indicate that the genetic approach as laid down by Dokuchaev, is the proper method for the study of soils.

REFERENCES

(1) Prasolov, L. I. 1928. Cartography of soils at the First International Congress of Soil Science held in Washington. *Pochvovedenie* 23 (1–2): 139–153.

(2) Tyurin, I. V. 1928. About the soils of North America. *Uchennuie Zap. Kazan. Gosudarstven. Univ.* 58: 27–48.

(3) Vilenskii, D. The principal features of the distribution of soils and vegetation in the United States and southern Canada. *Pochvovedenie* 23 (1–2): 99–138.

INTERNATIONAL ASSOCIATION OF FORESTRY EXPERIMENTAL STATIONS TO HOLD CONGRESS IN SWEDEN

At the invitation of the Swedish Institute of Experimental Forestry, silvicultural research workers and representatives of the Forestry Experimental Stations will hold a congress in Stockholm at the College of Forestry from July 22 to July 27, 1929.

The preliminary program lists the following subjects for discussions:

Reorganization of the International Association of Forestry Experimental Stations and the adoption of rules whereby it shall be governed.

Arrangement for an international bibliography of forestry.

Discussion on the question of the standardization of measuring methods and the investigation of experimental plots.

Discussion on methods and terminology in the sphere of forestry soil science.

Addresses describing the progress of silvicultural research.

During the congress and for a week preceding and a week following it, excursions will be conducted to different forest regions in Sweden.

The International Association of Forestry Experimental Stations was founded in 1891 for the purpose of discussing at international meetings and conferences questions of importance to the work of forestry research, of facilitating the scientific exchange of ideas between the different institutes, and, wherever possible, of working out standard methods for the carrying out of forestry investigations. The Stockholm congress will be the seventh meeting of the association.

Persons planning to attend the congress are requested to notify Arvid Lindman, Chairman of the Board of Governors, or Henrik Hesselman, Director of the Swedish Institute of Experimental Forestry, and to submit further proposals regarding subjects of discussion, at the same time mentioning any addresses which they may desire to give during the congress.

Enquiries and other communications concerning the congress should be addressed to Statens skogsförsöksanstalt, Experimentalfältet, Sweden.

Volume XXVII March, 1929 Number 3

SOIL SCIENCE

Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
Baltimore, Maryland, U. S. A.

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to Dr. Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

Soil Science is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Volumes begin with the January and July issues, respectively. The subscription price is $10.00 for Volumes XXV (Nos. 1–6 inclusive) and XXVI (Nos. 1–6 inclusive) January, 1928, to December, 1928, inclusive, United States, Mexico, Cuba. An additional charge per volume of 50 cents for subscriptions in other countries. *Back volumes* are supplied on orders for Vols. I to XIV inclusive, XIX to XXIV incl.—$100.00 per set.

New subscriptions are accepted to begin with the current issue, unless the subscriber gives instructions to begin the subscription with some other issue. If the subscriber desire, back issues of the current volume may be obtained when in stock. The publishers do not guarantee to supply back issues, however.

Renewal subscriptions. To avoid a break in your series, subscriptions should be renewed promptly on expiration. Otherwise it may prove impossible for the publishers to furnish the back issues missed, as only sufficient copies are printed of each issue to supply orders in hand.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; or G. Jervis Manton, 430 Lit. Collins St., Melbourne (and at Sydney and Brisbane)

For the British Empire, except Australia and Canada: Bailliere, Tindall & Cox; 8 Henrietta Street, Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann, Krausenstrasse 38, Berlin N. W., Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

Publishers of: *Medicine, Journal of Urology, Bulletin of the Johns Hopkins Hospital, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Physical Therapeutics, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thompson Research Laboratory, Journal of Biological Chemistry, Chemical Reviews. Journal of Dairy Science, Soil Science. Social Forces, Personnel Journal, Journal of Comparative Psychology. Comparative Psychology Monographs, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, American Speech, Childhood Education.*

RELATION OF TEMPERATURE TO THE AMOUNT OF NITROGEN IN SOILS

HANS JENNY

University of Missouri

Received for publication December 8, 1928

INTRODUCTION

In an earlier paper (9) it was shown that in semi-arid and semi-humid regions of the United States, the average nitrogen content of the soil increases two to three times for each 10°C. fall in mean annual temperature. This result agreed with a formula which was developed from the assumption that organic matter produced by vegetation and its destruction by microörganisms is primarily determined by climatic conditions. After a simple S-shaped curve is assumed for the decomposition process in respect to temperature, and when only soils of similar humidity and similar natural vegetation are considered, the equation takes the form:

$$N = \frac{a}{1 + e^{kt}} \tag{A}$$

where N represents the total nitrogen content of the soil in per cent, t the mean annual temperature, and a, k, constants.

Further material has been collected to support the results already secured. Five new curves are discussed in this paper; namely, curves for humid prairie and humid timber soils, and also curves for semi-humid prairie, terrace, and bottom land soils.

The nitrogen-temperature curves of these soil groups can all be described by the foregoing formula. In spite of this agreement, however, equation (A) represents no more than a first approximation of the true law. It may be considered as a working hypothesis leading to interesting and important practical results. Greater knowledge of the decomposition of organic matter with respect to temperature is needed and more soil analyses from northern latitudes should be secured in order to determine the absolute validity of the equation.

HISTORICAL

Few investigators have published definite statements concerning *functional* relationships of climate and soil nitrogen or soil organic matter. Of course, numerous scientists have assumed a climatic influence and supported their views with more or less convincing figures, obtained mostly from limited districts. Scientists who are working in regions of extreme climatic conditions,

for instance, in semi-deserts, tropics, the frigid zones, and high altitudes, are most interested in these quantitative relationships, since in these regions organic matter occurs either in excessive or deficient amounts, thus attracting particular attention.

Sievers and Holtz (28), on analyzing the soils in Washington, demonstrated that the total nitrogen content of the soil increases with altitude. They attributed this increase to the greater rainfall prevailing in the higher regions. Of much significance is J. C. Russell's (26) experimental curve for the prairie soils of Nebraska, which shows that the average nitrogen content increases logarithmically with increasing rainfall. Fortunately in this investigation temperature was kept nearly constant, although not intentionally so. DeTurk (2), summarizing a nitrogen survey of Illinois soils, expresses the idea that the low nitrogen content of the soils in the southern part of the state is due to the more favorable conditions for decomposition, such as higher temperature and rainfall. Attention should also be called to R. Lang's (12) deductions, although no data are given.

Very suggestive and directly related to this subject is the instructive paper of Senstius (27) who discusses the humus-temperature function in the tropical regions of Java.[1] Senstius, modifying a diagram of Mohr, also assumes an S-shaped curve for the destruction of organic matter. However, he combines regions of various types of vegetation thus complicating the mathematical treatment of the problem. He states with Mohr, as quoted from E. J. Russell (25); "Where the soil temperature is below 20°C., organic matter accumulates, being formed faster than it is decomposed; in the lowlands where the soil temperature exceeds 30°C., the decomposition of organic matter proceeds faster than its formation by vegetation, consequently there is no humus. At about 25°C. the soil humus remains constant." In high altitudes where the annual temperature is below 0°C. the microbiological activity is delayed and the surface soils contain from 20–30 per cent organic matter, in spite of the sparce vegetation (8).

SEMI-HUMID TERRACE SOILS

Semi-humid soils are characterized by the humidity factors[2] 280–380, the limits being, of course, somewhat arbitrarily fixed. One could also choose the annual precipitation-evaporation ratios of Transeau (13), but preference is given to the humidity factor, the application of which is not restricted to the United States alone.

The terracs, often styled "second bottoms" and "bench" lands, include old flood plains which now stand largely above the influence of overflow, the streams having cut their channels to lower levels. In their height above the first bottoms the terraces vary from a few feet to several hundred feet. There is

[1] This paper was not known to the author in 1927.

[2] Precipitation divided by saturation deficit of air (15).

often a series of distinct terraces arranged, one above another in step-like succession. The original vegetation consists mainly of timber. Figure 1 illustrates such conditions in Missouri.

The stream terraces are made up of alluvial material, consisting of deposits washed from slopes and uplands and deposited down stream by overflow waters. The source of this material is very heterogeneous, including wash from loessial, glacial, and a variety of residual soils, both timber and prairie. About 22 different soil series have been included, representing the states of Iowa (7), Missouri (19), and Arkansas (21).

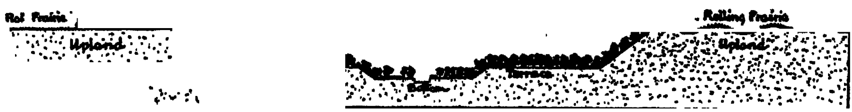


FIG. 1. SCHEMATIC ARRANGEMENT OF FLOOD PLAIN, TERRACE, AND UPLAND

The procedure of calculating the average nitrogen content of the terrace silt loam soils of a county is illustrated by the following example:

Black Hawk County, Iowa. Temperature 46.6°F. Humidity factor about 368

SOIL TQPE	PER CENT OF COUNTY AREA	N	AREA × N	AVERAGE N
		per cent		*per cent*
Waukesha silt loam	3.2	0.195	0.6240	0.7831
Bremer silt loam	0.6	0.242	0.1452	3.9
Calhoun silt loam	0.1	0.139	0.0139	0.201%N
Total	3.9		0.7831	

These county values (units) were classified according to their mean annual temperature class intervals of 2°F. being used. The mean nitrogen values of the various temperature classes were then plotted against the temperature midpoints of the classes and a curve was smoothed with the method of least squares, using the reciprocal mean errors as weights. The constant a was found by tryouts and then k was algebraically computed. The final formula has the expression:

$$N_{\text{Terrace}} = \frac{1.80}{1 + e^{0.069\,(t - 18.80)}} \qquad (B)$$

where N represents average nitrogen content of the soil in per cent and t the annual temperature F°. Observed and calculated values are summarized in table 1 and the graph is given in figure 2.

Also for terrace soils the N-T relation[3] is remarkably exponential. One

[3] Abbreviation of nitrogen-temperature relation.

wonders whether the uniformity in the distribution of nitrogen is entirely due to temperature or whether the nature of terrace soils consisting of outwash of surrounding upland soils which themselves show the N-T relation did not help to pronounce the relationship.

SEMI-HUMID BOTTOM LAND SOILS

The bottom land soils occur along the banks of the streams in continuous and interrupted strips varying from a few feet wide along the minor drainage courses to broad bottoms several miles wide. The broadest strip of strictly

TABLE 1

Nitrogen-temperature relation in semi-humid terrace soils (silt loams)

Humidity factors 280–380

MEAN ANNUAL TEMPERATURE		STATE	NUMBER OF COUNTIES (UNITS)	NUMBER OF NITROGEN VALUES	AVERAGE NITROGEN CONTENT	CALCULATED VALUE	DEVIATION
°F.	°C.				*per cent*		
44.0	6.67	Iowa	7	9	*0.322* ±0.056	0.269	+0.053
46.0	7.78	Iowa	2	4	*0.249* ±0.049	0.239	+0.010
48.0	8.89	Iowa	17	41	*0.198* ±0.007	0.212	−0.014
50.0	10.00	Iowa, Missouri	9	20	*0.181* ±0.011	0.187	−0.006
52.0	11.11	Iowa, Missouri	5	7	*0.160* ±0.020	0.165	−0.005
54.0	12.22	Missouri	5	8	*0.113* ±0.013	0.146	−0.033
56.0	13.33	Missouri	7	17	*0.095* ±0.015	0.128	−0.033
58.0	14.14	Missouri	3	8	*0.109* ±0.003	0.113	−0.004
62.0	16.67	Arkansas	4	4	*0.105* ±0.009	0.087	+0.018
64.0	17.78	Arkansas	2	2	*0.072* ±0.003	0.076	−0.004
Temperature range		Total	Total	Total	Nitrogen range	Constants	
20.0	11.11	3	61	120	0.322 to 0.072%	a = 1.80, k = 0.069	

alluvial land is along the Mississippi River near its confluence with the Arkansas River where the bottoms range from 75 to 100 miles wide. These first bottoms are subject to submergence by overflow waters. The surface is dominantly flat and level. Natural vegetation consists predominantly of timber with some meadows and swamps.

The source of all the material entering into the composition of alluvial soils cannot be precisely determined in all cases, but generally speaking, it consists of wash from loessial, glacial, residual, and terrace soils. The bottom land soils are immature because they are being added to by each overflow and also because the time between overflows is insufficient for and the conditions unfavorable to the advancement of those processes of weathering which have brought about

the different characteristics obtaining in older, better drained, normal soils. Oxidation has usually been inhibited by poor drainage (14).

Bottom land soils including 21 series in Iowa (7), Missouri (19), and Arkansas (21), were investigated. The same methods of arranging and computing the data were followed as in the case of terrace soils. Numerical information is given in table 3, graphic representation in figure 2. The equation has the form:

$$N_{\text{Bottom}} = \frac{1.60}{1 + e^{0.075\,(t - 24.00)}} \qquad (C)$$

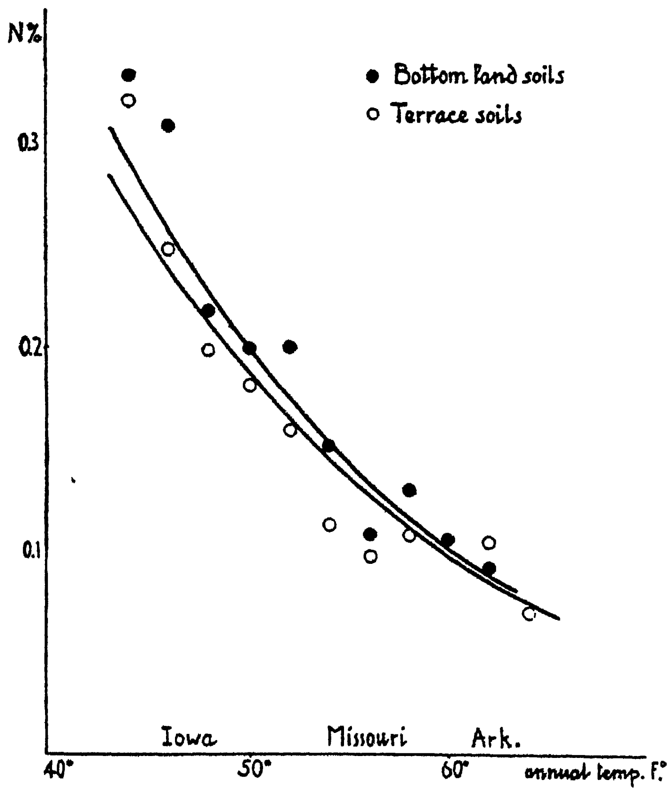


FIG. 2. NITROGEN-TEMPERATURE RELATION IN SEMIHUMID BOTTOM LAND SOILS (UPPER CURVE) AND TERRACE SOILS (LOWER CURVE) FOR SILT LOAMS

The curves of bottom land and terrace soils run nearly parallel, the former lying above the latter. The difference, however, is not important enough to indicate that bottom silt loams contain on the average more nitrogen than the terrace silt loams. The same similarity is observed for the carbon-nitrogen ratio, indicating that in both groups organic matter is equally well decomposed (table 2).

HUMID PRAIRIE AND TIMBER SOILS

East of the Mississippi River the precipitation-evaporation ratio increases slowly. The semi-humid region grades into the humid region. The part of humid region investigated includes southern Wisconsin, Illinois, western Kentucky, western Tennesee, and Mississippi. It is characterized by the humidity factors 300–420. The natural vegetation consists partly of timber and partly of prairie. Only upland soils, particularly silt loam soils, were studied. Fortunately the soil surveys of Wisconsin (32), Illinois (6), and Mississippi (5), separate clearly the prairie and timber soils in the classification, thus permitting the construction of two distinct curves for the entire region. As in the foregoing

TABLE 2

Carbon-nitrogen ratio in terrace and bottom soils of Iowa

TEMPERATURE		TERRACE SOILS (31 VALUES)	BOTTOM SOILS (31 VALUES)
°F.	°C.		
44.0	6.67	11.97 ±0.286	12.60 ±0.305
46.0	7.78	11.90 ±0.200	11.87 ±0.437
48.0	8.89	12.06 ±0.240	12.33 ±0.348
50.0	10.00	12.40 ± 0.235	11.88 ±0.463
Average		12.33 ±0.484	12.17 ±0.787

TABLE 3

Nitrogen-temperature relation in semi-humid bottom land soils (silt loams)

Humidity factors 280–380

MEAN ANNUAL TEMPERATURE		STATE	NUMBER OF COUNTIES (UNITS)	NUMBER OF NITROGEN VALUES	AVERAGE NITROGEN CONTENT	CALCULATED VALUE	DEVIATION
°F.	°C.				*per cent*		
44.0	6.67	Iowa	7	9	*0.327* ±0.040	0.292	+0.035
46.0	7.78	Iowa	2	2	*0.310* ±0.019	0.258	+0.052
48.0	8.89	Iowa	14	20	*0.218* ±0.020	0.227	−0.009
50.0	10.00	Iowa, Missouri	9	16	*0.200* ±0.012	0.199	+0.001
52.0	11.11	Iowa, Missouri	9	22	*0.202* ±0.016	0.175	+0.027
54.0	12.22	Missouri	12	24	*0.150* ±0.010	0.152	−0.002
56.0	13.33	Missouri	10	16	*0.109* ±0.008	0.133	−0.024
58.0	14.44	Missouri	4	11	*0.131* ±0.010	0.116	+0.015
60.0	15.56	Arkansas	3	7	*0.106* ±0.010	0.101	+0.005
62.0	16.67	Arkansas	2	6	*0.092* ±0.015	0.087	+0.005
Temperature range 18.0	10.00	Total 3	Total 73	Total 133	Nitrogen range 0.327 to 0.092%	Constants a = 1.60, k = 0.075	

examples the nitrogen values were arranged and computed. Figure 3, and tables 4 and 5 give the essential features. The equations have the form:

$$\underset{\text{Humid prairie}}{N} = \frac{1.60}{1 + e^{0.056\,(t - 18.20)}} \tag{D}$$

$$\underset{\text{Humid timber}}{N} = \frac{1.00}{1 + e^{0.062\,(t - 18.20)}} \tag{E}$$

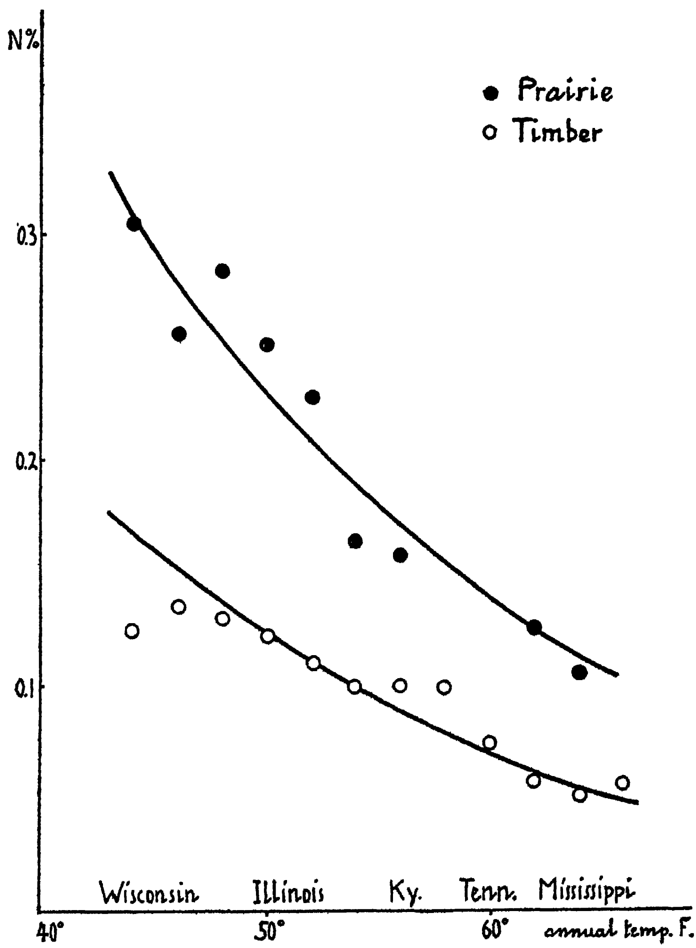


FIG. 3. NITROGEN-TEMPERATURE RELATION IN HUMID PRAIRIE (UPPER CURVE) AND HUMID TIMBER SOILS (LOWER CURVE) FOR SILT LOAMS

The nitrogen content of both prairie and timber soils decreases exponentially with increasing temperature, although this is less pronounced in the case of the timber curve. The well-known superiority of the prairie soils over the timber

soils is remarkably manifested for nitrogen in the entire region. The exact cause of this difference in nitrogen cannot be determined from the present data, since it is not known how much organic matter is produced by the forests of that region. The constants a in the equations (1.60, 1.00), which stand for the

TABLE 4

Nitrogen-temperature relation in humid prairie soils (silt loams)

Humidity factors 300–420

MEAN ANNUAL TEMPERATURE		STATE	NUMBER OF COUNTIES (UNITS)	NUMBER OF NITROGEN VALUES	AVERAGE NITROGEN CONTENT	CALCULATED VALUE	DEVIATION
°F.	°C.				*per cent*		
44.0	6.67	Wisconsin	2	6	*0.304* ±0.028	0.305	−0.001
46.0	7.78	Wisconsin Illinois	4	11	*0.256* ±0.011	0.287	−0.031
48.0	8.89	Illinois	9	9	*0.285* ±0.016	0.254	+0.031
50.0	10.00	Illinois	11	12	*0.252* ±0.009	0.231	+0.021
52.0	11.11	Illinois	8	10	*0.228* ±0.008	0.210	+0.018
54.0	12.22	Illinois	5	7	*0.164* ±0.012	0.190	−0.026
56.0	13.33	Illinois	2	3	*0.158* ±0.020	0.172	−0.014
62.0	16.67	Mississippi	5	16	*0.125* ±0.016	0.127	−0.002
64.0	17.78	Mississippi	6	19	*0.105* ±0.008	0.114	−0.009
Temperature range		Total	Total	Total	Nitrogen range	Constants	
20.0	11.11	4	52	93	0.304 to 0.105%	a = 1.60 k = 0.056	

TABLE 5

Carbon-nitrogen ratio in prairie and timber soils of Illinois (silt loams)

TEMPERATURE		PRAIRIE SOILS (33 VALUES)	TIMBER SOILS (32 VALUES)
°F.	°C.		
48.0	8.89	11.36 ±0.315	11.56 ±0.581
50.0	10.00	12.33 ±0.207	11.21 ±0.258
52.0	11.11	11.79 ±0.229	11.03 ±0.272
54.0	12.22	10.83 ±0.693	11.10 ±0.203
Average..............................		11.58 ±0.821	11.23 ±0.721

nitrogen content of the vegetation, suggest that the low nitrogen content of the timber might be partly responsible. It is also possible that the higher moisture content of the timber soils stimulates the decay of organic matter (4). The average carbon-nitrogen ratio is the same for both groups, as is illustrated in table 5.

NITROGEN-TEMPERATURE RELATION IN THE FLAT PRAIRIE SOILS OF MISSOURI

The question may arise as to how large an area must be considered in order to find a nitrogen-temperature relationship. Generally it can be answered that the narrower the annual isothermus run, the smaller the area that may lend itself to such studies.

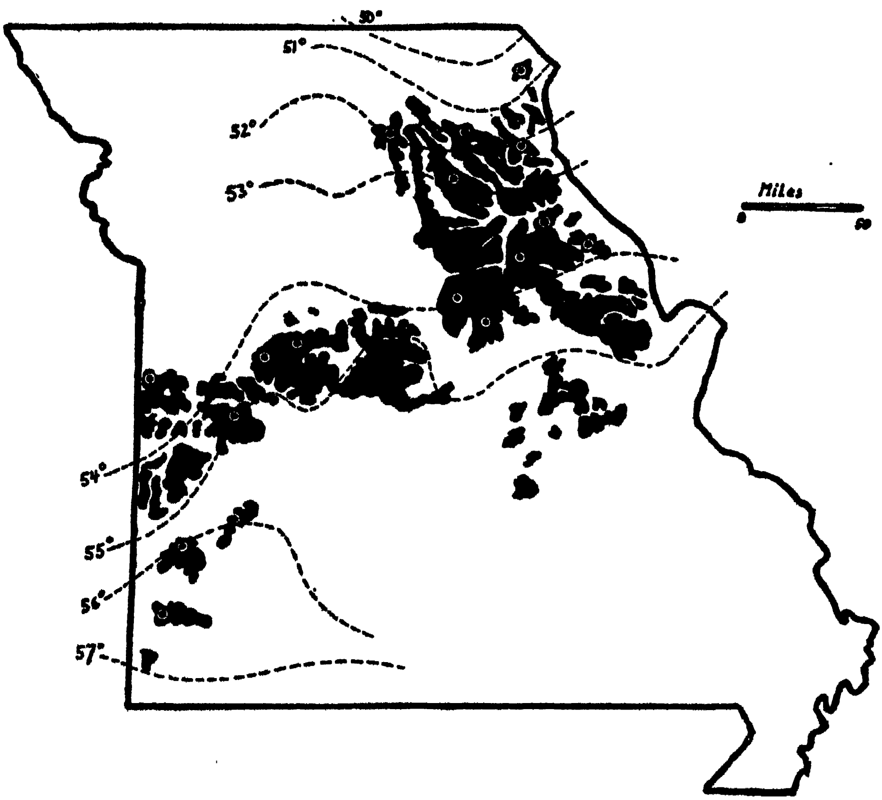


FIG. 4. LOCATION OF FLAT PRAIRIE SOILS IN MISSOURI AND ANNUAL ISOTHERMS IN DEGREES FAHRENHEIT (50°F. = 10.00°C. AND 57°F. = 13.89°C.)

To give a definite answer, the flat prairie soils of Missouri were selected, because they offer a minimum of variation in soil texture, profile, topography, drainage, vegetation, and humidity. The location of these prairie soils, including the soil types Putnam silt loam, Oswego silt loam, and Cherokee silt loam, is shown in figure 4. The area covered has a length from north to south (southern Iowa to southern Missouri) of about 280 miles and an annual temperature range of about 7°F. or about 4°C. (16). Climatic data of surrounding stations are given in table 7. The average nitrogen values for each county are given in

table 8, the graph in figure 5. With so narrow a temperature range it is safe to assume that the nitrogen-temperature relation can be represented by a straight line. After forming temperature classes with intervals of 2°F. and

TABLE 6

Nitrogen-temperature relation in humid timber soils (silt loams)

Humidity factors 300–420

MEAN ANNUAL TEMPERATURE		STATE	NUMBER OF COUNTIES (UNITS)	NUMBER OF NITROGEN VALUES	AVERAGE NITROGEN CONTENT	CALCULATED VALUE	DEVIATION
°F.	°C.				*per cent*		
44.0	6.67	Wisconsin	3	13	*0.124* ±0.010	0.168	−0.044
46.0	7.78	Wisconsin, Illinois	7	33	*0.135* ±0.008	0.151	−0.016
48.0	8.89	Illinois	9	9	*0.130* ±0.007	0.136	−0.006
50.0	10.00	Illinois	10	14	*0.124* ±0.005	0.122	+0.002
52.0	11.11	Illinois	8	12	*0.110* ±0.013	0.110	±0.000
54.0	12.22	Illinois	5	10	*0.099* ±0.007	0.098	+0.001
56.0	13.33	Illinois, Kentucky	4	9	*0.102* ±0.007	0.088	+0.014
58.0	14.44	Illinois, Kentucky, Tennessee	9	15	*0.100* ±0.009	0.080	+0.020
60.0	15.56	Tennessee, Mississippi	5	14	*0.074* ±0.008	0.069	+0.005
62.0	16.67	Mississippi	5	12	*0.057* ±0.006	0.062	−0.005
64.0	17.78	Mississippi	7	20	*0.051* ±0.003	0.055	−0.004
66.0	18.89	Mississippi	3	20	*0.056* ±0.012	0.051	+0.005
Temperature range		Total	Total	Total	Nitrogen range	Constants	
22.0	12.22	5	75	185	0.135 to 0.051%	a = 1.00	k = 0.062

TABLE 7

Climatological data of stations surrounding the Missouri flat prairie soils

STATION	ALTITUDE		MEAN ANNUAL TEMPERATURE		HUMIDITY FACTOR
	feet	*meters*	°F.	°C.	
Keokuk, Iowa	614	187	52.3	11.28	336
St. Joseph, Mo.	967	294	54.0	12.22	261
Columbia, Mo.	784	239	54.5	12.50	354
Kansas City, Mo.	963	293	54.8	12.67	276
Springfield, Mo.	1,224	344	55.6	13.11	352
St. Louis, Mo.	567	173	56.0	13.33	291

nitrogen classes with intervals of 0.02 per cent N, a correlation coefficient (r) of −0.77 can be computed. It expresses the degree to which the two variables, nitrogen and temperature, tend to be associated. Perfect correlation would

give a value of −1.00; no correlation, a value of zero. With the aid of the correlation coefficient the line of best fit can easily be calculated. It has the form:

$$N = -0.0131\, t_{49°}^{57°} + 0.8522$$

where N represents the average nitrogen content of the soil in per cent and t the mean annual temperature in °F. (49°F.–57°F.).

Summarizing we may state that the N-T relation can be clearly demonstrated within a region as small as that covered by the flat prairie soils of Missouri.

TABLE 8

Nitrogen temperature relation in the flat prairie soils of Missouri

Humidity factors 261–354

MEAN ANNUAL TEMPERATURE		COUNTY	NUMBER OF NITROGEN VALUES	AVERAGE TOTAL NITROGEN CONTENT	SOIL TYPE
°F.	°C.			*per cent*	
49	9.44	Wapello, Ia.	1	0.222	Putnam silt loam
51	10.56	Clark, Mo.	1	0.158	Putnam-like Grundy
52	11.11	Knox, Mo.	4	0.178	Putnam silt loam
52	11.11	Harrison, Mo.	3	0.206	Putnam silt loam
52.3	11.28	Lee, Iowa	1	0.177	Putnam silt loam
53	11.67	Rolls, Mo.	3	0.119	Putnam silt loam
53	11.67	Shelby, Mo.	8	0.169	Putnam silt loam
53.4	11.89	Cass, Mo.	1	0.133	Oswego silt loam
53.5	11.94	Marion, Mo.	10	0.145	Putnam silt loam
53.5	11.94	Macon, Mo.	9	0.157	Putnam silt loam
53.5	11.94	Pike, Mo.	1	0.135	Putnam silt loam
53.7	12.06	Audrien, Mo.	4	0.142	Putnam silt loam
54.4	12.44	Pettis, Mo.	6	0.152	Oswego silt loam
54.5	12.50	Boone, Mo.	1	0.118	Putnam silt loam
54.9	12.72	Callaway, Mo.	3	0.155	Putnam silt loam
55	12.78	Johnson, Mo.	2	0.161	Oswego silt loam
55.6	13.06	Henry, Mo.	8	0.121	Cherokee silt loam
56.4	13.56	Barton, Mo.	1	0.100	Cherokee silt loam
57.2	14.00	Newton, Mo.	1	0.122	Cherokee silt loam

The carbon-nitrogen ratio

Equation A for nitrogen should hold as well for oganic matter expressed as total organic carbon. One has only to put C in the place of N in the formula and adjust the constants. The approximate amount of organic carbon can be found by multiplying the percentage of nitrogen by 10, which is based upon the fact that in temperate regions the carbon-nitrogen ratio in the soil is about 10 (31). This ratio seems to vary somewhat with temperature, since it is wider in the north than in the south, as shown in table 9 and figure 6. Because of the limited amount of data, both the semi-arid and semi-humid regions were included (3, 7, 10, 26, 29).

The functional change of the C/N ratio seems to support the general character of equation A, which demands that under low temperatures the nitrogen content of the soil approaches the nitrogen content of the vegetation (constant a) and consequently, the C/N ratio of the soil tends to become as wide as that of the undecomposed organic material.

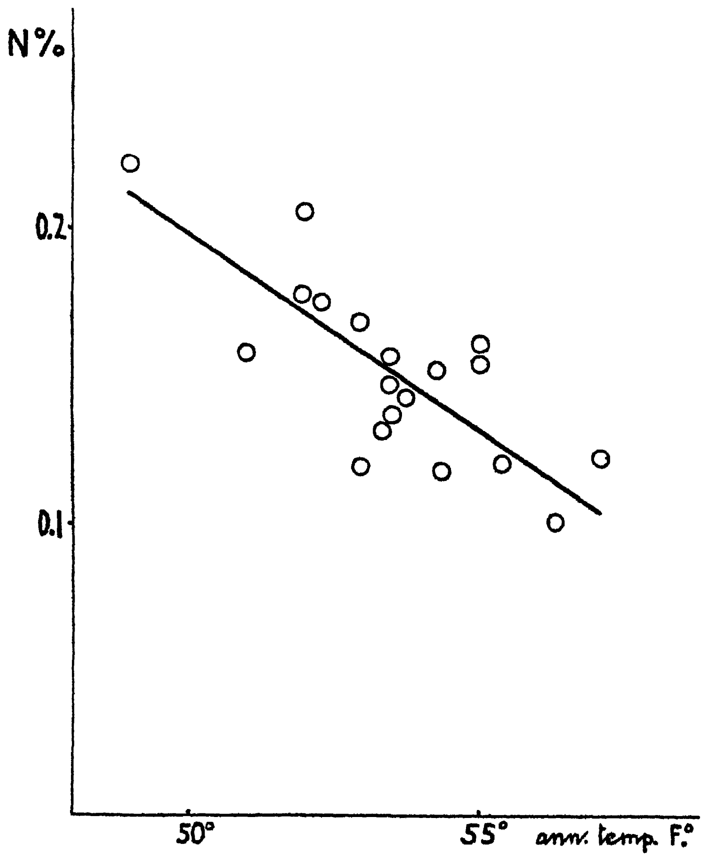


FIG. 5. NITROGEN-TEMPERATURE RELATION OF THE FLAT PRAIRIE SOILS OF MISSOURI

DISCUSSION

Temperature

Whether the nitrogen temperature equation is expressed in units Fahrenheit or Centigrade or in absolute units starting with −273°C., does not matter, as they can easily be converted. More important is the choice of the meteorological unit, since no soil temperatures are available. Shall the nitrogen content be related to the annual temperature, to the number of months or days having

temperatures above the freezing point, or to some other criterion of the heat effect, such as frost, free season, or daily temperature summations?

TABLE 9

Carbon-nitrogen ratio and temperature (loams and silt loams)

TEMPERATURE		STATE	C/N	NUMBER OF VALUES (COUNTIES)
°F.	°C.			
35.0	1.67	Saskatchewan (Canada)	13.25 ±0.555	6
44.0	6.67	Iowa	12.21 ±0.210	10
46.0	7.78	Iowa	12.30 ±0.122	4
48.0	8.89	Iowa	11.85 ±0.191	19
50.0	10.00	Iowa Nebraska	12.11 ±0.136	14
52.0	11.11	Nebraska	11.90 ±0.383	4
54.0	12.22	Nebraska Kansas	11.15 ±0.470	6
57.0	13.89	Kansas	10.30 ±0.681	3
68.0	20.00	Texas	9.1	76

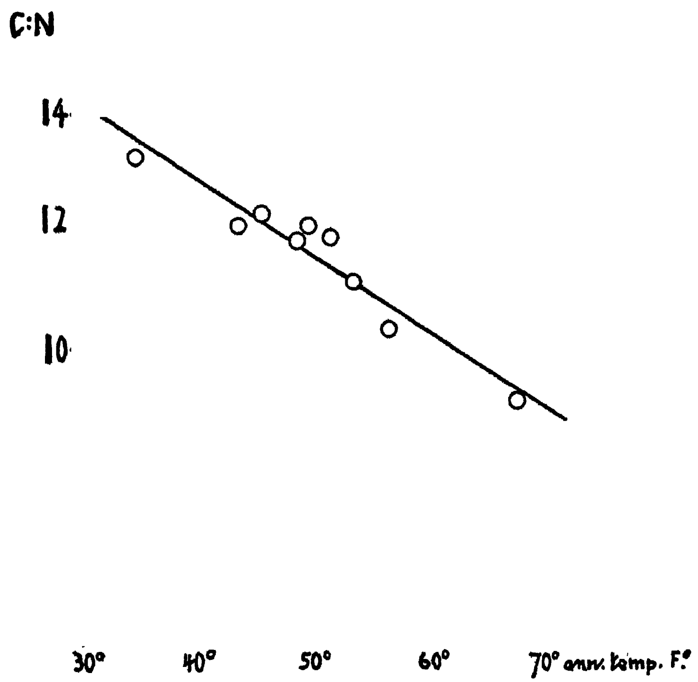


Fig. 6. Carbon-Nitrogen Ratio and Temperature in Grassland Soils

The mean annual temperature was chosen because it is the easiest value to obtain from meteorological stations (30). Its use, however, is restricted to regions having similar marches of annual temperatures. That this is the case in the states investigated is shown by figure 7, where the annual march of temperature in the latitudes 29° to 49° is illustrated (Kincer).

The deviation of the nitrogen values

The dots in the various N-T diagrams represent averages of their respective temperature classes. The question naturally arises as to how widely the single values are scattered about the average. Are these class-means reliable enough to be put in a function of temperature? Information is obtained from the mean error (or probable error, which is 0.6745 times the mean error) for the class-

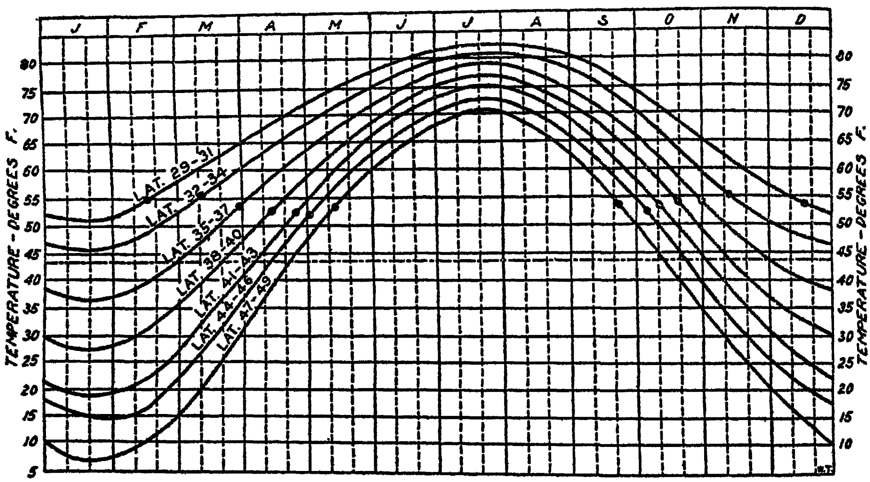


FIG. 7. ANNUAL MARCH OF TEMPERATURE OF THE INVESTIGATED REGIONS (AFTER KINCER)

averages, and from the correlation-ratio for the deviation of the values about the curve. The mean error was calculated according to the common formula:

$$m \qquad \overline{n(n-1)}$$

where m represents mean error, n total number of values, and Σv^2 the sum of the squares of the numbers formed by subtracting each value from the arithmetical mean of the whole class.

As an index of the deviation of the single nitrogen values (county units) about the curve, the correlation ratio was chosen (18). It measures the degree of relationship between the single nitrogen values and the corresponding temperature in so far as this relationship may be described by a curve passing through the mean of every temperature class. If the relationship is ideal, if there is no

scatter about the curve fitted in this way, η will have a value of 1. If there is no relationship, if the scatter about the curve is as great as the dispersion about the mean of all nitrogen values, η will have a value of zero. The formula used is

$$\eta = \sqrt{1 - \frac{\sigma_{NT}^2}{\sigma_N^2}}$$

TABLE 10

Correlation-ratio of the various soil regions

CURVE	η
Semi-arid region, grassland	0.91
Semi-humid upland soils (predominating prairie)	0.90
Semi humid terrace soils (predominating timber)	0.78
Semi-humid bottom soils (timber)	0.78
Humid upland soils (prairie)	0.89
Humid upland soils (timber)	0.84
Flat prairie soils of Missouri	0.77

TABLE 11

Nitrogen-temperature relation in the semi-arid region

Humidity factors 125–250

MEAN ANNUAL TEMPERATURE		STATE	NUMBER OF COUNTIES (UNITS)	NUMBER OF NITROGEN VALUES	AVERAGE NITROGEN CONTENT	CALCULATED VALUE	DEVIATION
°F.	°C.				*per cent*		
32[a]	0.0	Canada	....	10	*0.44*[b]	0.344	+0.096
36	2.22	Canada, N. Dakota	5	30	*0.295* ±0.050	0.297	−0.002
38	3.33	N. Dakota	4	5	*0.291* ±0.032	0.276	+0.015
40	4.44	N. Dakota	5	12	*0.244* ±0.015	0.256	−0.012
42	5.56	N. Dakota	1	1	*0.21*[b]	0.237	−0.027
48	8.89	Nebraska	2	17	*0.199* ±0.0077	0.187	+0.012
50	10.00	Nebraska	7	28	*0.192* ±0.012	0.172	+0.020
55	12.78	Kansas	6	47	*0.152* ±0.022	0.140	+0.012
58	14.44	Texas	12	32	*0.100* ±0.0065	0.124	−0.024
60	15.56	Texas	3	8	*0.081* ±0.022	0.114	−0.033
64	17.78	Texas	3	27	*0.085* ±0.012	0.096	−0.011
66	18.89	Texas	2	40	*0.112* ±0.010	0.088	+0.024
68	20.00	Texas	3	25	*0.063* ±0.0091	0.081	−0.018
70	21.11	Texas	13	48	*0.075* ±0.0076	0.075	−0.000
72	22.22	Texas	5	18	*0.075* ±0.0040	0.068	+0.007
Temperature range		Total	Total	Total	Range	a = 1.70	
40°	22.22°	5	71	348	0.44 to 0.063%	k = 0.045	

[a] Temperature estimated.

[b] Not used in calculating the constants.

Where η represents correlation ratio, σ_{NT} the standard deviation of all single nitrogen values about a line passing through the mean of the various temperature classes, and σ_N the standard deviation of all single nitrogen values about their arithmetical average. This correlation ratio of the various soil regions is listed in table 10. Generally speaking, the correlation ratio is about 0.85, indicating that a nitrogen-temperature relation is pronounced.

The deviation of nitrogen values may be due to errors in sampling (variations in depth) or to factors which hasten or delay the decomposition process in the

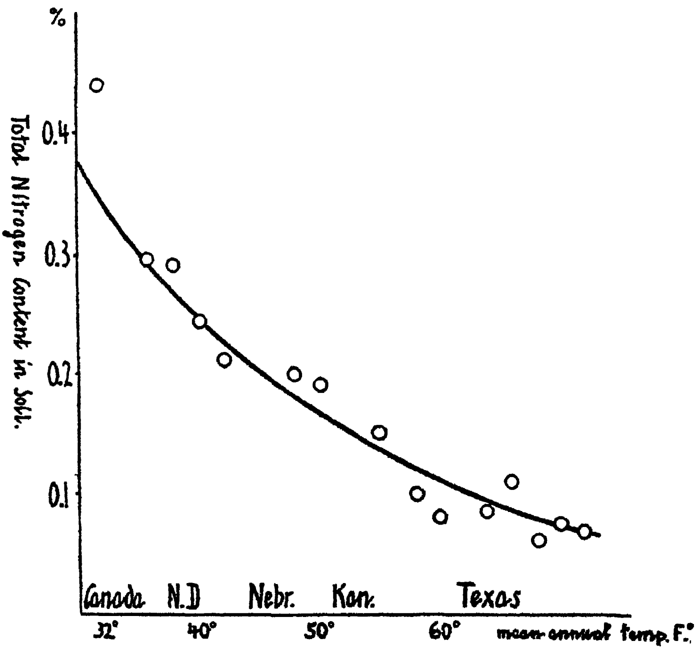


FIG. 8. AVERAGE TOTAL NITROGEN CONTENT OF THE SOIL AS RELATED TO THE MEAN ANNUAL TEMPERATURE IN THE SEMI-ARID REGION

field above or below "normal." Such factors are physical and chemical properties of the soil, age, topography, geological origin, soil climate, vegetation, and cultivation.

Nitrogen turn-over

Virgin prairie soils have a nitrogen content of about 20 to 40 per cent higher than that of cultivated prairie soils which have been cropped for 30 to 40 years. A simultaneous decrease of fertility is demonstrated by the rapidly decreasing yields of untreated experiment plots.

Often the idea is expressed that nitrogen and organic matter should be

restored by adding sufficient amounts of green manure and stable manure. In several publications a nitrogen content of 0.250 to 0.300 per cent nitrogen is given as a desirable value for a fertile soil. This figure corresponds to the nitrogen content of Minnesota, Iowa, and northern Illinois prairie soils.

TABLE 12

Nitrogen-temperature relation in the semi-humid region (upland soils)

Humidity factors 280–380

MEAN ANNUAL TEMPERATURE		STATE	NUMBER OF COUNTIES (UNITS)	NUMBER OF NITROGEN VALUES	AVERAGE NITROGEN CONTENT	CALCULATED VALUE	DEVIATION
°*F.*	°*C.*				*per cent*		
32[a]	0.00	Canada	......	16	*0.475*[b]	0.455	+0.020
34	1.11	Canada	1	17	*0.393* ±0.023	0.414	−0.021
36	2.22	N. Dakota	7	20	*0.341* ±0.017	0.376	−0.035
38	3.33	N. Dakota	4	6	*0.341* ±0.083[b]	0.340	+0.001
40	4.44	N. Dakota	10	32	*0.295* ±0.017	0.307	−0.012
42	5.56	Minnesota	7	17	*0.273* ±0.018	0.276	+0.003
44	6.67	Minnesota	16	44	*0.266* ±0.012	0.248	+0.018
46	7.78	Minnesota Iowa	11	23	*0.266* ±0.014	0.222	+0.044
48	8.89	Iowa	9	26	*0.210* ±0.014	0.198	−0.012
50	10.00	Iowa Missouri	21	54	*0.172* ±0.0050	0.177	−0.005
52	11.11	Iowa Missouri	11	60	*0.168* ±0.0070	0.160	+0.008
54	12.22	Missouri Kansas	10	56	*0.150* ±0.012	0.140	+0.010
56	13.33	Missouri	13	56	*0.101* ±0.0094	0.124	−0.023
58	14.44	Missouri Arkansas	9	20	*0.098* ±0.0095	0.110	−0.012
60	15.56	Arkansas	8	8	*0.091* ±0.0081	0.098	−0.007
62	16.67	Arkansas	3	3	*0.078* ±0.0057	0.086	−0.008
66	18.89	Louisiana	3	6	*0.056* ±0.0015	0.067	−0.011
68	20.00	Louisiana	4	11	*0.050* ±0.0064	0.059	−0.009
Temperature range		Total	Total	Total	Range	a = 1.55	
36°	20.00°	9	147	475	0.475 to 0.050%	k = 0.065	

[a] Temperature estimated.

[b] Not used in calculating the constants.

From the nitrogen temperature curves it is obvious that the building up of nitrogen in the soil meets greater difficulties the farther south one goes. As a matter of fact 20 to 30 years of manuring experiments in southern states have failed to secure any considerable increase in soil nitrogen. Even as far north as Wooster, Ohio (mean annual temperature 49°F.), it has not been possible to reach the original nitrogen content of the unbroken soil (22).

Recently the term "nitrogen turn-over" has been suggested, a term which refers to the amount of nitrogen that may be supplied to crops from rotation to rotation by means of crop residues, green manures, and farm manures (17).

Results of earlier work

For the sake of completeness two curves and tables previously obtained are included. Table 11 and figure 8 refer to soils of various textures of the semi-

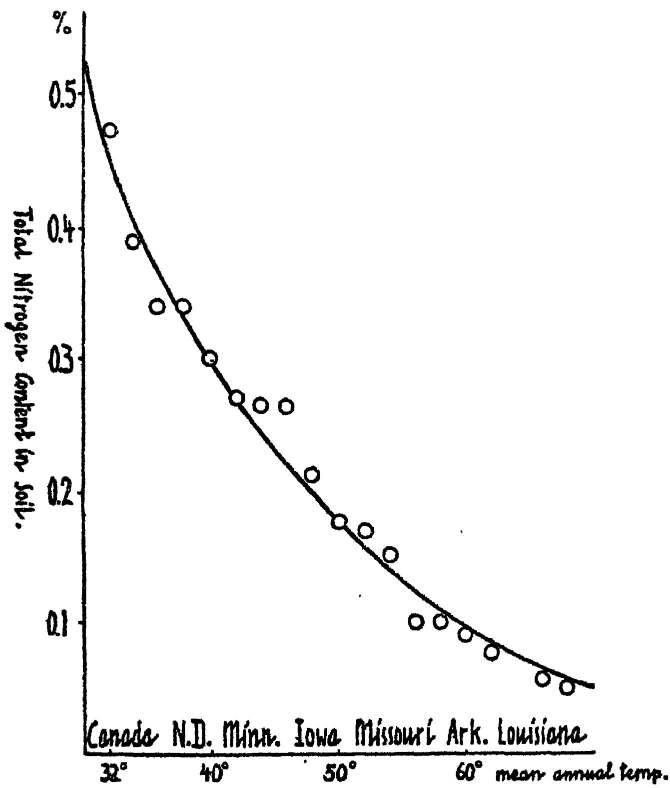


Fig. 9. Average Total Nitrogen Content of the Soil as Related to the Mean Annual Temperature in the Semi-Humid Region (Upland Soils)

arid grassland region lying within the Great Plains. Table 12 and figure 9 show the N-T relation in the semi-humid region for upland soils (loams and silt loams). The original predominating vegetation is tall prairie grass. The values above 50°F. (which were not used in calculating the constants) represent soils with original forest vegetation mainly of oaks. These values are somewhat below the theoretical grass land values for that part of the curve.

SUMMARY

1. In the semi-arid, semi-humid, and humid regions of the United States a correlation exists between the mean annual temperature and the average total nitrogen content of upland prairie and timber soils and of terrace and bottom land soils.

2. The decrease of nitrogen with increase of temperature is exponential or, in other words, the logarithm of the nitrogen varies inversely to the temperature.

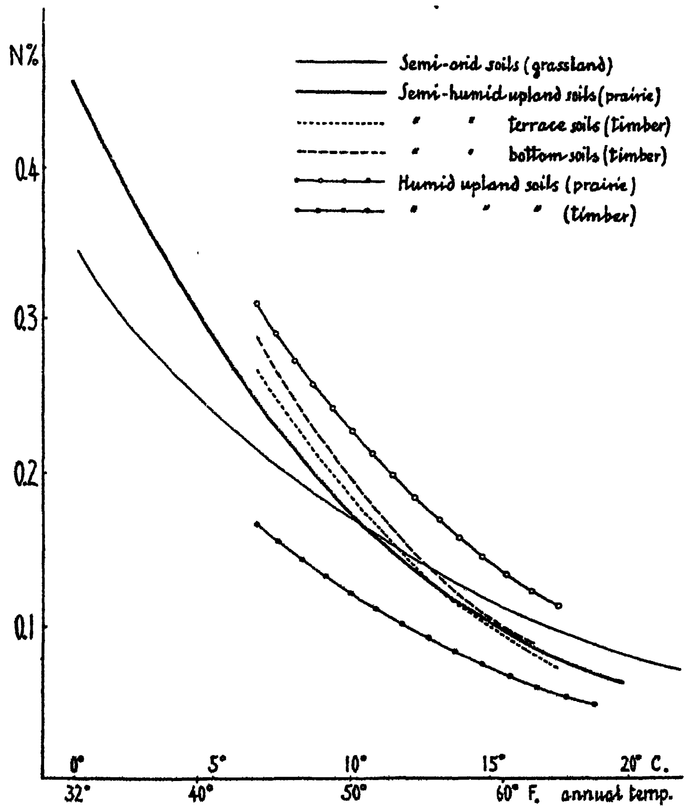


FIG. 10. GRAPHIC SUMMARY OF NITROGEN-TEMPERATURE RELATIONS

Generally speaking, for every 10°C. decline in mean annual temperature, the average nitrogen content of the soil increases two to three times (fig. 10).

3. The carbon-nitrogen ratio of the soil organic matter seems to become narrower with increasing temperature.

REFERENCES

(1) AVERITT, S. D. 1915 The soils of Kentucky. Agr. Exp. Sta. Bul. 193.

(2) DETURK, E. E. 1922 The relation between soil type and chemical composition. Rept. 3rd Ann. Meet. Amer. Soil Survey Assoc. Bul. 4, Vol. 2, 130–149.

(3) FRAPS, G. S. 1922 Organic constituents of the soil. Texas Agr. Exp. Sta. Bul. 300.
(4) FULLER, G. D. 1914 Evaporation and soil moisture in relation to the succession of plant associations. *Bot. Gaz.* 58:193–234.
(5) HUTCHISON, W. L., AND PERKINS, W. R. 1900 Soils of Mississippi. Miss. Agr. Exp. Sta. Bul. 65.
(6) Illinois Agricultural Experiment Station Soil Reports No. 1–39, 1911–1927.
(7) Iowa Agricultural Experiment Station Soil Survey Reports No. 1–42, 1917–1926.
(8) JENNY, H. 1926 Die alpinen Böden. In: Braun—Blanquet, J. unter Mitwirkung von Jenny, H: Vegetations entwicklung und Bodenbildung in der alpinen Stufe der Zentralalpen. *Denkschriften der Schweiz. Naturf. Ges.* 63 (2): 295–340.
(9) JENNY, H. 1928 Relation of climatic factors to the amount of nitrogen in soils. *Jour. Amer. Soc. Agron.* 20: 900–912.
(10) JOEL, A. H., MITCHELL, T., AND EDMONDS, F. H. 1926 Soil survey of the Bienfait—Oxbow Area, Sackatchewan. Soil Report No. 5, Saskatoon. Canada.
(11) KINCER, J. B. 1919 Relation between vegetative and frostless periods. *U. S. Dept. Agr. Mo. Weather Rev.* 47: 106–110.
(12) LANG, R. 1920 Verwitterung und Bodenbildung als Einführung in die Bodenkunde. E. Schweizerbart, 'sche Verlagsbuchhdlg., Stuttgart.
(13) LIVINGSTON, E., AND SHREVE, F. 1921 The distribution of vegetation in the United States of America as related to climatic conditions. Washington, D. C.
(14) MARBUT, C. F. ET AL. 1913 Soils of the United States. U. S. Dept. Agr. Bul. 96.
(15) MEYER, A. Uber einige Zusammenhänge zwischen Klima und Boden in Europa. *Chem. der Erde* 1926: 209–347.
(16) MILLER, M. F., AND KRUSEKOPF, H. H. 1918 The soils of Missouri. Mo. Agr. Exp. Sta. Bul. 153.
(17) MILLER, M. F., AND KRUSEKOPF, H. H. 1927 The rate of accumulation and cost of nitrogen and carbon in soils under different systems of green manuring and cropping. *Mo. Agr. Exp. Sta. Bul.* 256: 86–87.
(18) MILLS, F. C. 1924 Statistical methods. New York.
(19) Missouri, Unpublished data.
(20) MOOERS, C. A. 1906 The soils of Tennessee. Tenn. Agr. Exp. Sta. Bul. v. 19, no. 4, whole no. 78.
(21) NELSON, M., SACHS, W. H., AUSTIN, R. H. 1923 The soils of Arkansas. Ark. Agr. Exp. Sta. Bul. 187.
(22) Ohio Agricultural Experiment Station *Ann. Rpt.* 1927 *Bul.* 402: 15–22.
(23) PETER, A. M., AND AVERITT, S. D. 1906 Soils. Ky. Agr. Exp. Sta. Bul. 126.
(24) PIETERS, A. J. 1927 Green Manuring. New York.
(25) RUSSELL, E. J. 1926 Plant Nutrition and Crop Production. Univ. Calif. Press.
(26) RUSSELL, J. C., AND MCRUER, WM. 1927 The relation of organic matter and nitrogen content to series and type in virgin grassland soils. *Soil Sci.* 24: 421–452.
(27) SENSTIUS, M. W. 1925 The formation of soils in equatorial regions, with special reference to Java. *Amer. Soil Survey Assoc. Rpt. Bul.* 6, 1: 149–161.
(28) SIEVERS, F. J., AND HOLTZ, H. F. 1923 The influence of precipitation on soil composition and on soil organic matter maintenance. Wash. Agr. Exp. Sta. Bul. 176.
(29) SWANSON, C. O. 1914 Chemical analyses of some Kansas soils. Kans. Agr. Exp. Sta. Bul. 199.
(30) U. S. Department of Agriculture. 1926 Weather Bureau, Bul. W., ed. 2.
(31) WAKSMAN, S. A. 1924 Influence of microorganisms upon the carbon-nitrogen ratio in the soil. *Jour. Agr. Sci.* 14: 555–562.
(32) WHITSON, A. R. 1927 Soils of Wisconsin. Wis. Geol. and Nat. Hist. Survey Bul. 68, Soil Ser. 49.

THE RELATION BETWEEN CONCENTRATIONS OF POTASSIUM IN CULTURE SOLUTIONS AND OPTIMUM PLANT GROWTH[1]

R. P. BARTHOLOMEW AND GEORGE JANSSEN[2]

Arkansas Agricultural Experiment Station

Received for publication November 19, 1928

Potassium fertilizers at the present time are applied largely because of soil needs. Some attempt has been made to consider the needs of the plants, as determined by their chemical analyses. The different crops have been grouped into general classes in accordance with their need for large amounts of potassium (alfalfa and sweet clover) or for only small amounts (oats and wheat). Although such groupings are undoubtedly very helpful in making recommendations for potassium fertilizers, they are too general to be of much value. Moreover, although they establish the approximate total amount of potassium needed by the plants they do not give any index as to the amount of available potassium which must be present in solution in the nutrient media during the life of the plant.

It is well known that plants immediately use only soluble constituents. Hence it might be logical to assume that the composition of the soil solution should give some indication as to the amounts of potassium available for plant nutrition. Reports of several investigations showed a wide variation in the potassium content of the soil solution. Hibbard (13), working with alkali soils, found variations from 21 p.p.m. to 2110 p.p.m. of potassium in the soil solution. Millar (20), from analyses of displaced solutions from soils of the humid region, reports variations of 2.5 p.p.m. to 6.2 p.p.m. potassium for silt loam soils, 1.4 p.p.m. to 2.6 p.p.m. for sands, and 15.3 p.p.m. to 41.0 p.p.m. for peats. Unfortunately we have no good method for determining the rate of availability, and the figures given above show only the initial concentration when the amount in solution is in equilibrium with the relatively insoluble compounds.

If a knowledge of the minimum concentration of potassium required for the maximum growth of a plant could be combined with a knowledge of the concentration of potassium in the soil solution and the ability of the insoluble compounds to maintain that concentration, a good basis for potash fertilization could be established.

The work reported in this paper had for its object the solution of the following

[1] Published with the approval of the director of the Arkansas Agricultural Experiment Station. Published as Research Paper No. 114, Journal Series, University of Arkansas.

[2] Assistant agronomists.

questions: 1. What are the minimum concentrations of potassium essential for plant growth? 2. Will plants assimilate more potassium than the amount required for optimum growth?

MINIMUM POTASSIUM REQUIREMENTS

In a preliminary report the writers (3) noted that there was considerable variation in the concentrations of potassium necessary to obtain maximum growth of the different crops. Parker and Pierre (22) report from the results of their experiments that the maintenance of a concentration of 2 p.p.m. of potassium would probably be sufficient for the best growth of corn and soybeans.

PLAN OF EXPERIMENT

The seeds were germinated in quartz sand which contained very little, if any, of the soluble plant-food constituents. When the plants were two to three inches high they were transferred to culture solutions. The plants were placed through one-inch holed in paraffined boards and held in place by means of cotton plugs. The boards were then placed over 2-gallon jars filled with nutrient solution containing different amounts of potassium.

IMPORTANCE OF PROPER NUTRIENT SOLUTION

The selection of the proper nutrient solutions for studies of the requirements of plants for any one element is of great importance. If any element were included in the solution which might have some indirect effect on another element it would greatly impair the value of the results obtained.

Many of the standard nutrient solutions contain some compounds of sodium. Because of the similarity of the chemistry of sodium and potassium the question arose as to the effect of the presence of sodium on the assimilation of potassium by plants.

A review of the literature on the value of sodium in plant nutrition was made in order to determine whether the presence of sodium in a nutrient solution would greatly affect the results of a study of the potassium requirements of plants. Doll (7) grew barely in pot cultures composed of one-third sandy soil and two-thirds pure river sand fertilized with sodium chloride, potassium chloride, and mixtures of the two. He concluded that sodium chloride was only slightly less effective than potassium chloride for the nutrition of crops. Gerlach (8) concluded from field experiments that sodium can partially replace potassium when the amounts of potassium are insufficient for normal plant growth. Hartwell (9) from solution experiments with wheat seedlings, obtained a 10 per cent increase in yield from an application of sodium chloride. He concluded that certain functions of potassium in plants can be performed by sodium. On the other hand, he claims that certain functions of potassium can not be performed by sodium and without sufficient potassium for these needs, maximum growth will not be obtained. Somewhat similar results are reported

by Hartwell and Damon (10) from field experiments. Pfieffer (23) states that if there is a deficiency of potassium for the whole plant, sodium will replace potassium in the leaves and stalks and make the potassium available for the grain. Kruger (18), from the results of pot experiments with beets, concludes that sodium can not physiologically replace potassium although its presence permits plants to use increased quantities of potassium more readily. Atterberg (1), from experiments with oats, thought that potassium was partially replaced by sodium. Hoagland (14) and Breazeale (6) concluded that the presence of sodium depressed the intake of potassium by plants. Reed and Haas (24), who studied the effect of sodium on the growth of young orange trees, concluded that sodium can not replace potasssium in plant processes. The trees fertilized with sodium made good growth but the authors decided that the potassium had been transferred from the roots to the leaves. Jordan and Genter (16) also concluded, from the results of pot experiments, that sodium can not perform the function of potassium. Stahl and Schroeder (26) hold the same view. They concluded from the results of pot cultures in which they grew buckwheat, carrots, peas, and oats, on potassium deficient peat, that the increased yields from the sodium treatment over the no treatment jars were insignificant. The foregoing does not cover a complete review of the literature, but sufficient evidence is presented to show that the presence of sodium in a nutrient solution in which the potassium requirements of plants are to be studied may give erroneous results.

Because of the fact that many of the standard nutrient solutions, such as Shive's (25), Knop's (17), and Tottingham's (27) contain sodium, or contain potassium in combinations which are not readily adjusted for varying the concentrations, a suitable nutrient solution had to be devised. The first nutrient solution used was a modified form of that used by Hartwell and Wheeler (11) and contained the following salts: Solution I: $Ca(NO_3)_2 \cdot 4H_2O$, 147 gm.; $CaH_4(PO_4)_2 \cdot H_2O$, 26.9 gm.; dissolve and make to 2280 cc. Solution II: $MgSO_4 \cdot 7H_2O$, 90 gm.; dissolve and make to 2280 cc.

To each jar, containing 7.5 liters of distilled water, 50 cc. each of solutions I and II were added. Preliminary experiments with this nutrient solution and the one reported in the following, has shown that the concentrations of salts indicated were more than sufficient to maintain normal growth during the intervals between renewals, when sufficient potassium was added to the solution. Therefore in the experiments reported hereafter, potassium was the only element limiting plant growth, and any increase in the amount of dry matter produced by increasing the concentrations of potassium can be attributed only to the increase in the concentration of potassium.

The potassium was added as needed from two solutions of KCl. One solution contained 28.62 gm. of pure KCl per 2000 cc. This was equivalent to 14.997 gm. of K per 2000 cc., or 0.0075 gm. of K per cc. When 1 cc. of this solution was diluted to 7500 cc. the concentration became 1 mgm. of K per liter, or 1.0 p.p.m. The other solution contained 2.862 gm. of pure KCl per

2000 cc. This concentration is equivalent to 0.00075 gm. of K per cubic centimeter. One cubic centimeter of this solution diluted to 7500 cc. produced a concentration of 0.1 mgm. of K per liter or 0.1 p.p.m. The concentration of potassium used varied from 0 mgm. to 50 mgm. of K per liter of nutrient solution. In further discussion, in order to eliminate repetition, the term "concentration of so many p.p.m. of K," means mgm. of K per liter of nutrient solution.

Oats and alfalfa grew very well on this solution. Soybeans and tomatoes grew well for a while but eventually became very chlorotic. This affected the growth to such an extent that the results could be considered as of only a qualitative nature. After several attempts to grow soybeans and tomatoes in this solution failed, several different solutions were tried with varying results. The one finally adopted was one based on the solutions devised by Hoagland and Martin (15) and Parker (21). The solutions used were: Solution A: 807 gm. $Ca(NO_3)_2 \cdot 4H_2O$, 143 gm. $Mg(NO_3)_2 \cdot 6H_2O$, 5 gm. $CaCl_2$, 4 gm. $MgCl_2$, 1 gm. H_3BO_3, water to make 2 liters; Solution B: 300 gm. $MgSO_4 \cdot 7H_2O$, 1.2 gm. $MnSO_4$, water to make 2 liters; Solution C: 7gm. $CaH_4(PO_4)_2 \cdot H_2O$, water to make 2 liters.

Three separate solutions are necessary to prevent precipitation of some of the constituents. Ten cubic centimeters each of solutions A, B, and C were added to 7.5 liters of distilled water. This concentration, as has also been demonstrated by Parker (21), was more than sufficient to supply the needs of the plants for these elements during the periods between renewal of the concentration of these minerals.

The potassium was added from two solutions of KCl of the concentration previously given. The potassium concentration was renewed twice a day according to the following method. Two jars were filled with distilled water and the nutrient solutions, with the exception of potassium, added. The plants were then transferred from the old solution containing no potassium. Sufficient potassium as potassium chloride was added in solution to bring the concentration of the old no potassium treatment to 0.5 p.p.m. of potassium. The solution was well mixed and aerated by stirring with a wooden paddle. To the old 0.5 p.p.m. jars an amount of potassium chloride equivalent to 0.5 p.p.m. was added and it became the new 1.0 p.p.m. jar to which the 1.0 p.p.m. plants were then transferred. Similarly, amounts equivalent to 1 p.p.m. of potassium were added to the old 1 p.p.m. jars and they became the 2 p.p.m. jars. A similar process was repeated until all the plants had been transferred to a renewed potassium concentration. The same kind of procedure was employed every time the solutions were changed. By using this procedure the potassium concentration was renewed twice a day and all other nutrients, excepting iron, were renewed once every three days.

It was impossible to keep the concentrations exactly up to standard by this procedure because of the feeding of the plants. However, as there are no methods of chemical analysis for the rapid determination of potassium, the

procedure mentioned above would probably be as accurate as analyzing the solutions once every few days and adding the same amount each day, assuming that the daily potassium consumption between periods of analyses was uniform.

In order to add sufficient iron for normal growth the following procedure was adopted. Every third day the pH of the jars containing no potassium was reduced to 4.0 by the addition of dilute hydrochloric acid, and 50 cc. of a 0.5 per cent solution of ferric tartrate added. Therefore as the plants were rotated for the different potassium concentrations they received the necessary amount of iron. In this way no plant was kept in a solution of pH 4.0 for over 12 hours and the possibility of injurious action due to such an acid reaction was reduced. The plants were all grown until they started to blossom, when they were harvested and first air-dried, then oven-dried, and then weighed.

The crops used in this study were oats, alfalfa, Hubam clover, cowpeas, soybeans, Sudan grass, and cotton. In order to reduce as much as possible the

TABLE 1

Oven-dry weights of plants grown in nutrient solutions with concentrations of potassium indicated

K IN SOLUTION	OATS, WEIGHT OF 4 PLANTS		ALFALFA WEIGHT OF 4 PLANTS		HUBAM CLOVER, WEIGHT OF 12 PLANTS	COWPEAS WEIGHT OF 8 PLANTS		SOY-BEANS, WEIGHT OF 8 PLANTS	SUDAN GRASS, WEIGHT OF 12 PLANTS		COTTON, WEIGHT OF 8 PLANTS
	Tops	Roots	Tops	Roots	Tops	Tops	Roots	Tops	Tops	Roots	Tops
p.p.m.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.0	1.0	0.7	0.26	0.07	0.85	5.5	2.4	3.75	5.3	1.4	4.6
0.5	10.0	3.3	3.40	1.60	1.90	24.9	8.3	11.85	45.1	6.1	8.0
1.0	10.9	3.5	2.70	1.20	1.71	30.6	9.4	10.05	53.4	5.2	9.3
2.0	14.5	4.8	2.80	1.40	1.72	35.1	10.2	14.10	43.3	8.3	12.4
3.0	9.8	3.3	3.20	1.70	1.75	32.8	9.4	13.65	71.1	7.2	12.7
5.0	7.1	2.2	2.90	1.40	1.95	43.0	13.2	15.45	72.0	13.8	13.3
10.0	11.9	2.9	......	......	......	......	......	......	......	......	......
25.0	10.8	2.8	......	......	......	......	......	......	......	......	......
50.0	10.6	3.2	......	......	......	......	......	......	......	......	......

error which might be caused by plant variation, the plants were grown at the rate of two to six plants per jar, depending on the crop. Because of the differences in the plants used the different ones will be discussed separately.

OATS

The oats grew very rapidly after being transferred to the culture solutions. They tillered very profusely and were harvested after growing in the culture solutions for 50 days. The yields are given in table 1.

There is no definite correlation between the root growth and the top growth. In a very general way the larger top growths are accompanied by somewhat larger root growths, but because the correlation is so general, only the weight of the tops is considered in determining the minimum needs of the plant.

Oats are generally considered as having a low total potassium requirement and yet the maintenance of a concentration of 2 p.p.m. of potassium seems to be essential for the best growth. Increasing the concentrations up to 50 p.p.m. of potassium did not cause any additional growth. Very good growth was obtained at low concentrations. A concentration of only 0.5 p.p.m. of potassium produced two-thirds as much growth as the best concentration of 2 p.p.m.

ALFALFA AND HUBAM CLOVER

Alfalfa and Hubam clover are both regarded as needing large amounts of potassium for normal growth and for that reason will be discussed together. The alfalfa had grown 59 days in the nutrient solutions when it was harvested and the Hubam clover had grown 56 days. The oven-dry weights are given in table 1. As in the case of oats, the correlation between the weights of tops and roots of alfalfa is so general that only the weights of the tops will be considered in determining the minimum requirement of alfalfa for potassium.

Both alfalfa and Hubam clover seem able to make maximum growth if a concentration of 0.5 p.p.m. of soluble potassium is maintained. An increase in the concentration of soluble potassium up to 5 p.p.m. did not produce any larger amount of dry matter. The ability of these two crops to make such good growth on such a low concentration of potassium in contrast to the large amounts usually found in alfalfa and Hubam clover hay suggests that these crops may have great ability to feed upon the relatively insoluble soil minerals. That these plants can feed readily upon relatively insoluble potassium containing minerals such as feldspars has already been demonstrated by Bauer (4).

COWPEAS

The growth of cowpeas was very luxuriant, and marked evidence of physiological disturbances was only found in the plants receiving no potassium in their nutrient solution. The leaves turned to a purplish brown color about two weeks after these plants were transferred to the culture solutions, and made very little additional growth by the time of harvesting. The cowpeas were grown in the nutrient solutions for 38 days after which they were harvested. The results are given in table 1.

As in the preceding experiments, because of the very general correlation between the weights of tops and roots, only the dry weight of the top growth will be considered in determining the potassium requirement of cowpeas.

Cowpeas, according to the analyses given by Henry and Morrison (12), have a much higher total requirement for potassium than does alfalfa. In contrast to alfalfa, which was able to make its maximum growth on a concentration of 0.5 p.p.m. of potassium, the minimum concentration of potassium necessary to produce best growth of cowpeas appears to be very close to 2 p.p.m. This suggests a very marked difference in their ability to feed upon relatively insoluble potassium compounds and indicates that cowpeas, although having a

relatively high total potassium requirement, are unable to secure their minimum needs from very dilute solutions of potassium. However, very good growth was obtained at a concentration of 0.5 p.p.m. of potassium. In fact from the growth of the plants (pl. 1, fig. 1) it was practically impossible to see much difference in the plants growing in concentrations of from 1 to 5 p.p.m. of potassium. Part of the increase in weight of the plants growing in the solutions having a concentration of 5 p.p.m. of potassium was due to an extra 48 hours growth for special observations in connection with work of another problem.

SOYBEANS

The increasing importance of soybeans as a soil building and hay crop makes desirable some information in regard to its potassium requirement. For this reason soybeans were included in the crops studied. The technique used in growing these plants was the same as that described for the preceding crops. The plants started growing rather slowly after being transferred to the culture solutions, but after about a week they apparently recovered and made good growth. Forty-eight days after being transferred to the culture solutions the plants were harvested. The yields are given in table 1.

From the analyses given by Henry and Morrison (12) soybeans do not require nearly as much total potassium for best growth as do cowpeas and yet the maintenance of a concentration of approximately 2 p.p.m. of potassium was necessary for the production of best plant growth. Although a little more growth was produced at a concentration of 5 p.p.m. of potassium, it was considered due to plant variation, because the dry weights from concentrations of 2 and 3 p.p.m. of potassium were approximately the same. As with preceding crops, fairly good growth was obtained at a concentration of 0.5 p.p.m. of potassium without any indication of physiological disturbances which might be attributed to a deficiency of potassium.

SUDAN GRASS

Since Sudan grass does not have a very high potassium requirement, a comparison of it with crops such as alfalfa and cowpeas, which have high potassium requirements, may be of some value in studying the minimum concentrations necessary to produce good growth of different crops. Twelve plants per treatment were grown, in order to eliminate as far as possible differences in yields due to plant variations. The plants were very vigorous and made rapid growth after being transplanted to the culture solutions. After 38 days, pictures were taken of the plants and they were harvested. The results are given in table 1.

As probably might be expected, the correlation between dry weight of tops and roots was very general. On the whole it may be said that a large increase in dry weight of tops was usually accompanied by an increase in the dry weight of the roots.

From the dry weights of the tops, Sudan grass seems to require the maintenance of a higher concentration of potassium than do any of the preceding crops. The maintenance of a concentration of close to 3 p.p.m. of potassium seems to be necessary for the best growth of Sudan grass. However, good growth was obtained (pl. 1, fig. 2) in all solutions to which potassium had been added. The stems were very rigid and did not need any support to prevent breaking of the plants. About two-thirds as much growth was made with a concentration of 0.5 p.p.m. potassium as was obtained at the best concentration, namely, 3 p.p.m. of potassium. No evidence of any physiological disturbance such as might be caused by a deficiency of potassium was observed. The failure of 2 p.p.m. of potassium to produce any more growth than 0.5 p.p.m may be due to some extent to plant variation.

COTTON

More fertilizer is used on cotton than on any other crop, and a knowledge of its potassium requirement would give a better understanding for proper fertilization. The cotton plants, Trice 323, were grown as in the preceding experiments. They started growing rather slowly, after being transferred to the culture solutions, but after three weeks began to grow at what might approximate average rate of growth in the field. At the end of 81 days the plants were harvested. The dry weights are given in table 1.

The results indicate that the requirement of cotton is similar to that for cowpeas and that the maintenance of a concentration of potassium of approximately 2 p.p.m. is necessary for best growth.

This is rather interesting when it is considered that cotton has a relatively low potassium requirement as compared to cowpeas, and it may be one of the reasons why cotton responds so readily to applications of fertilizers. The growth at a concentration of 0.5 p.p.m. was not as good in proportion to that obtained from the higher concentrations as was found with the preceding crops.

POTASSIUM CONTENT OF PLANTS

It has been reported from a number of sources that plants seem able to take up more potassium than is actually needed for growth. One of the writers (2) has reported results that indicate a large consumption of potassium by alfalfa, oats, and Sudan grass above their actual needs. Blair (5) states that certain crops such as soybeans are able to take up an excess of potassium when there is an abundant supply. Lende (19) reports that plants will take up more potassium than they need for growth. If all plants will take up more potassium than needed for normal growth, it will not only put a new interpretation upon potassium fertilization but may be of value in helping to explain the ability of different plants to feed upon potassium compounds.

Analyses of potassium content were made on the plant tissue, as follows:

Weighed samples of plant tissue were saturated with H_2SO_4 and ignited.

The ash was extracted with water and made to a definite volume. Aliquots were evaporated to dryness with a little H_2SO_4 and then ignited. After cooling, a few cubic centimeters of water was added and the silica and iron were removed by filtration. The sulfates and any remaining iron and aluminum were removed with barium hydroxide. The barium was then removed by the addition of carbon dioxide and then filtering. Platinic chloride was added to the filtrate and the potassium weighed as potassium platinic chloride. The results of the analyses are given in table 2. The potassium content of the oat tops at a concentration of 10, 25, and 50 p.p.m. of potassium was 1.96 per cent, 3.52 per cent, and 3.33 per cent respectively.

As a general rule there was a gradual increase in the percentage of the potassium in the plant when the concentration of the potassium increased. The most noteworthy exception was the Hubam clover where the potassium content of the plants was approximately the same for plants grown in solutions containing 1, 2, 3, and 5 p.p.m. of potassium. Even in this case, however, there

TABLE 2

Percentages of potassium in plant tissues grown in nutrient solutions with concentrations of potassium indicated

K IN SOLUTION	K IN OATS TOPS	K IN ALFALFA TOPS	K IN HUBAM CLOVER TOPS	K IN COWPEAS TOPS	K IN SOYBEANS TOPS	K IN SUDAN GRASS TOPS	K IN COTTON TOPS
p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.0	....	....	1.01	0.31	....	0.40	0.78
0.5	1.22	1.76	1.76	0.71	1.13	0.52	0.81
1.0	1.34	1.54	2.67	0.81	1.63	0.68	1.18
2.0	2.40	2.17	2.58	1.08	1.77	0.92	1.10
3.0	2.61	1.90	2.71	1.20	2.21	0.84	1.48
5.0	3.03	2.24	2.60	1.34	1.97	1.07	1.80

was 50 per cent more potassium in the plants grown in a concentration of 1 p.p.m. than there was in plants grown in a concentration of 0.5 p.p.m. potassium, which was the minimum amount necessary for best growth. With alfalfa, the plants grown in a concentration of 1 p.p.m. potassium had a smaller percentage of potassium than the plants grown in 0.5 p.p.m., which was the minimum concentration necessary for best growth. All the other concentrations contained a considerably higher percentage of potassium. Cowpea, soybean, Sudan grass, cotton, and oat plants grown on concentrations of potassium greater than that required for normal growth, with the exception of plants grown in a concentration of 10 p.p.m. of potassium, all contained a considerably higher percentage of potassium than was found in the plants grown in the concentrations necessary for optimum growth.

The results of the analyses of Hubam clover and oats suggest that there is a maximum amount of potassium which plants will assimilate. In the case of Hubam clover the addition of potassium above a concentration of 1.0 p.p.m. failed to increase the percentage of potassium in the plant. With oats there

was an increase in potassium content of the plants up to a concentration of 25 p.p.m. of potassium in solution. Increasing the concentration to 50 p.p.m. of potassium had no effect upon the percentage of potassium in the plant. The assimilation of available potassium in amounts equivalent to the maximum capacity of plants would play a very important part in determining the residual effects which could be expected from applications of potassium fertilizers. However, the results are only suggestive and further study is necessary to determine whether all plants have a maximum capacity for the assimilation of potassium.

GENERAL DISCUSSION

The results of the experiments have shown that there is a decided difference in the concentrations of potassium necessary to produce best growth. Alfalfa and Hubam clover required the maintenance of a concentration of only 0.5 p.p.m. of potassium to make best growth, whereas cowpeas, oats, soybeans, and cotton required the maintenance of a concentration of 2 p.p.m. for the most favorable growth. To produce the best yield, Sudan grass required the maintenance of a higher concentration than any of the above crops—3 p.p.m.

There was no correlation between the total potassium requirements of the plants and the minimum concentration which must be maintained to secure best growth. For example, cowpeas, alfalfa, and Hubam clover are regarded as crops requiring large total amounts of potassium for normal growth and yet a concentration of only 0.5 p.p.m. was sufficient, when maintained, to completely satisfy the needs of the latter two crops, whereas the maintenance of a concentration of 2 p.p.m. of potassium was essential for the best growth of cowpeas. Soybeans with a moderate total requirement for potassium, and oats with a relatively low total potassium requirement both need the maintenance of a concentration of 2 p.p.m. of potassium in order to make the best growth. On the other hand, Sudan grass, which is regarded as having a low total potassium requirement, needed the maintenance of approximately 3 p.p.m. of potassium for best growth.

The differences in the concentrations of potassium necessary to produce best growth of plants may offer some explanation for the differences in the ability of plants to produce good growth from difficultly soluble potassium compounds. The results of these studies suggest that both alfalfa and Hubam clover should be able to feed very heavily upon slightly soluble potassium minerals such as orthoclase feldspar. This is in excellent agreement with results presented by Bauer (4), who secured normal growth of sweet clover when feldspar was the only source of potassium. On the other hand oats, cowpeas, soybeans, cotton, and Sudan grass would not be expected to feed very heavily upon relatively insoluble potassium minerals because they require much larger concentrations for best growth.

The cause of the difference in potassium requirements of the plants could not be determined from these experiments. Part of it might be explained from

the theory advanced by Truog (28), that variations in the internal acidity of the plants greatly affect the ability of the plant to feed upon potassium. According to his theory, plants, such as alfalfa and sweet clover, whose sap has a slightly acid to alkaline reaction should do very well upon small concentrations of potassium while other crops having a more acid sap would require greater concentrations of potassium for normal growth. It is doubtful, however, whether the foregoing explanation would account for the intake of potassium above the amount necessary for minimum growth. More work is necessary to determine the cause of the assimilation of excessive amounts of potassium by plants and the action, if any, of the excess of potassium in the plant.

In all of the experiments good growth was obtained at a concentration of approximately 0.5 p.p.m. of potassium, and no evidence of physiological disturbances due to a deficiency of potassium was observed. Apparently the symptoms supposedly characteristic of potassium starvation develop only when the concentration of soluble potassium is extremely small.

Data, to be published in the near future, from studies with tomato plants show that the potassium in the plants is very labile. In other words, if there is a deficiency of potassium for normal growth in an embryonic region, sufficient potassium will be translocated from a region of older tissue to permit the plant to function almost normally in the embryonic region. When the deficiency of available potassium becomes very great, however, physiological disturbances will be observed in the older regions.

From a consideration of the foregoing facts, it seems reasonable to believe that when physiological disturbances which may be attributed to a deficiency of potassium are observed in the embryonic regions, the amount of potassium available for plant growth is practically nil.

The assimilation of large amounts of potassium by plants from solutions of greater concentration than that required for maximum growth will enable plants, because of the lability of the potassium assimilated, to function normally when the concentration in solution is reduced below that essential for best growth.

The ability of plants to take up excessive amounts of potassium may determine to a great extent the residual effect which may be expected from the application of a potassium fertilizer. If the potassium remains fairly available after being applied to the soil, most of that added in the fertilizer may be removed by the first crop and the residual affect may be rather small, if any.

From the standpoint of fertilizer practices it would appear to be almost necessary to make approximately the same application of potassium fertilizers for all crops. Although many of the crops may not use all of the potassium added, the larger application seems to be essential to maintain a concentration large enough to secure best growth of those requiring a concentration of approximately 2 to 3 p.p.m. of potassium.

SUMMARY

In order to determine the minimum concentration of potassium which is necessary to produce optimum growth, oats, alfalfa, Hubam clover, cowpeas, soybeans, Sudan grass, and cotton were grown in culture solutions having different concentrations of potassium. Analyses were made on the plant tissue to determine whether plants have the ability to assimilate more potassium than that required for optimum growth. The results of the experiments may be briefly summarized as follows:

1. There was considerable variation in the requirements of different plants. Alfalfa and Hubam clover were able to make optimum growth in a solution containing 0.5 p.p.m. of potassium. Oats, cowpeas, soybeans, and cotton made best growth at a concentration of 2 p.p.m. potassium while Sudan grass required a concentration of 3 p.p.m. in order to produce the best growth.
2. All the plants made good growth at a concentration of 0.5 p.p.m. potassium.
3. There was no relation between total potassium requirements of plants and the concentration necessary to produce optimum growth. For example, oats and cowpeas have a low and high total potassium requirement respectively, and yet both needed a concentration of 2 p.p.m. of potassium to make optimum growth.
4. Plants absorbed more potassium than is required for optimum growth.

REFERENCES

(1) Atterberg, A. 1891. Sodium as a plant nutrient. *Deut. Landw. Presse.* 1891: 1035. Abs. in *Exp. Sta. Rec.* 3:554.

(2) Bartholomew, R. P. 1928. The availability of potassium to plants as affected by barnyard manure. *Jour. Amer. Soc. Agron.* 20: 55–81.

(3) Bartholomew, R. P., and Janssen, G. 1927. The rôle of potassium in plant nutrition. *Ark. Agr. Exp. Sta. Bul.* 221: 63.

(4) Bauer, F. C. 1921. The relation of organic matter and the feeding power of plants to the utilization of rock phosphate. *Soil Sci.* 12: 21–41.

(5) Blair, A. W. 1919. Utilizing soil potash by means of intermediary crops. *Proc. Soc. Prom. Agr. Sci.* 39: 69–74.

(6) Breazeale, J. F. 1906. The relation of sodium to potassium in soil and solution cultures. *Jour. Amer. Chem. Soc.*, 28: 1013–1025.

(7) Doll, P. 1902. On the potash fertilizing of barley and the replacement of potash by soda. *Landw. Vers. Stat.* 57: 471–476.

(8) Gerlach, M. 1901. The action of kainit and high-percentage potassium salts. *Fühling's Landw. Ztg.* 50: 377–388, 409–416.

(9) Hartwell, Burt, L. 1908. Sodium as a partial substitute for potassium. *R. I. Agr. Exp. Sta. Ann. Rpt.* 1908: 242.

(10) Hartwell, B. L., and Damon, S. C. 1919. The value of sodium when potassium is insufficient. *R. I. Agr. Exp. Sta. Bul.* 177.

(11) Hartwell, B. L., Wheeler, H. H., and Pember, F. R. 1907. The effect of additions of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. *R. I. Agr. Exp. Sta. Ann. Rpt.* 1907: 229.

(12) Henry, W. A., and Morrison, F. B. 1915. Feeds and Feeding. Madison, Wis.

(13) Hibbard, P. L. 1923. Comparison of the soil solution by displacement method and the water extracts of alkali soils. *Soil Sci.* 16: 465.

(14) Hoagland, D. R., and Martin, J. C. 1923. A comparison of sand and solution cultures with soils as a media for plant growth. *Soil Sci.* 16: 372.

(15) HOAGLAND, D. R., AND MARTIN, J. C. 1923. Effect of salts on the intake of inorganic elements and on the buffer system of the plant. *Cal. Agr. Exp. Sta. Tech.* Paper 8.
(16) JORDAN, W. H., AND GENTER, C. G. 1901. The substitution of soda for potash. *N. Y. Agr. Exp. Sta. Bul.* 192: 333–350.
(17) KNOP, W. 1868. Der Kreislauf des Stoffes. Lehrbuch der Agriculturchemie, p. 572–663. Leipzig and St. Petersburg.
(18) KRUGER. 1914. Can sodium wholly or partially replace potassium as a fertilizer for sugar beets? *Ztscher. ver Deut. Zuckerindus.* 703: 697–792. Abs. in *Exp. Sta. Rec.* 32: 230.
(19) LENDE, N. J. 1912. Presumed luxury consumption of potassium and phosphoric acid. *Meddel. Norske. Myrselkap.* 5: 65.
(20) MILLAR, C. E. 1923. Studies on virgin and depleted soils. *Soil Sci.* 16: 433–448.
(21) PARKER, F. W. 1927. Soil phosphorus studies. III. Plant growth and the absorption of phosphorus from culture solutions of different phosphate concentrations. *Soil Sci.* 24: 129–147.
(22) PARKER, F. W., AND PIERRE, W. H. 1928. The relation between the concentration of mineral elements in a culture medium and the absorption and utilization of these elements by plants. *Soil. Sci.* 25: 337–343.
(23) PFEIFFER, T. ET AL. 1905. Experiments on the assimilation of potash and soda by plants. *Mitt. Landw. Inst. Breslau.* 3: 567–613.
(24) REED, H. S., AND HAAS, A. R. C. 1923. Studies on the effects of sodium and potassium and calcium on young orange trees. *Cal. Agr. Exp. Sta. Tech.* Paper 11.
(25) SHIVE, J. W. 1915. A study of physiological balance in nutrient media. *Physiol. Res.* 1: 327.
(26) STAHL, M., AND SCHROEDER, P. 1898. Potash and soda in plants. *Jour. Landw.* 47: 49–84.
(27) TOTTINGHAM, W. E. 1914. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* 1: 133.
(28) TROUG, E. 1922. The feeding power of plants. *Science* 41: 294–298.

PLATE 1

FIG. 1. Growth of cowpeas in nutrient solution with parts per million of potassium indicated.

FIG. 2. Growth of Sudan grass in nutrient solution with parts per million of potassium indicated.

FIG. 1

FIG. 2

A CRITICAL STUDY OF THE INFLUENCE OF SOIL TYPE ON THE CALCIUM AND MAGNESIUM CONTENT AND OTHER PHYSIOLOGICAL CHARACTERS OF THE ALFALFA PLANT[1]

JOHN F. FONDER[2]

Michigan State College

Received for publication November 19, 1928

Alfalfa is so important in the rations of all classes of farm animals, and its use in the maintenance and improvement of soil fertility has become so extensive that some knowledge, both of the degree to which its composition is variable and of the effect of soil differences upon its composition, is of unquestioned value.

Up to the present time considerable work has shown that a number of plants vary in composition when grown under different soil conditions and when given different fertilizer treatments. One of the important phases of this work has been in connection with the effect of different soil conditions upon the composition of the expressed juice of the plants.

It was to obtain information of the foregoing nature, in regard not only to the alfalfa plant but to several of the other important legumes, that this study was undertaken. This paper includes data showing the variations in the calcium and magnesium content of alfalfa stems and leaves, and likewise of their expressed juices, at different ages when grown on different soil types. In addition to these it contains data bearing on other relationships of the alfalfa plant and soil type.

HISTORICAL

McCool (14) has shown that the addition of fertilizers to soils under field conditions causes a measurable increase in the concentration of the expressed sap of corn plants, of sugar beets, of table beets, and of onions, and to a less extent of table carrots. These data also showed that there was nearly as much variation among the concentrations of the sap of young corn plants grown on different soil types, as there was among those grown on fertilized and unfertilized plots. The addition of fertilizers as noted by Austin (2) altered the composition of soybeans. The greatest effect was on Coloma sand and the least on Miami loam. But it was also observed that soil type was of greater

[1] A part of a thesis presented to the graduate committee of the Michigan State College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his appreciation to Dr. M. M. McCool for his kindly interest in helping to plan and organize the work presented here.

influence in determining the composition of the soybean plants than was the application of fertilizers. McCool and Weldon (16) observed that the addition of fertilizers caused no evident increase in the amounts of calcium and magnesium present in the juice of wheat grown on Hillsdale sandy loam, but that the application of lime alone gave greater amounts of calcium present in the expressed juice. It was concluded by these authors that the application of the various nutrients to the soil generally resulted in an increase of these elements in the plant juice. Experiments carried out by Morse (19) seem to indicate less CaO in the plant tissue of clover, on a dry basis, grown on limed soils than on unlimed. On the other hand, Bryan (3) found that the calcium content of the tissue of alfalfa, alsike clover, and red clover grown in sand cultures was higher when the reaction of the nutrient solution was higher. McCool (15), states that the composition of the roots and tops of alfalfa varies according to soil fertility, stage of development, and varieties grown. It was concluded by Gilbert and Hardin (7) that the amounts of mineral elements in plant solutions were closely correlated with the amounts of these added in fertilizers.

Dinsmore (6) found, as alfalfa advanced in growth, a rather wide fluctuation in total ash content, although the tendency was to decrease as the plants became more mature. This was borne out by Widstoe (23), who showed also that there was a greater amount of water in the stems than in the leaves of alfalfai n the early stages of growth but that as the plants become older it was nearly the same in the stems and leaves or even greater in the leaves. The dry matter was greater in the leaves in the beginning but toward maturity became much less than in the stems. In second cutting alfalfa, the ash content was found by Widstoe (23) to be lower than that of the first cutting. Austin (2) found the calcium and magnesium content of soybeans to decrease as the plants became older and that the magnesium content was not so uniform as the calcium content. Burd (4) showed that in barley there were probably three periods of growth: one, a preliminary period of about six weeks in which the greatest gain in weight occurred; then a period of about six weeks in which the rate of gain in total weight became negligible; and a third period, continuing until maturity, in which there was an actual loss in weight, not only in the whole plant but in the various parts as well. However, it was observed further that the increase in dry matter followed a fairly straight line throughout the life of the plant and that the increase of calcium and magnesium parallel the formation of dry matter up to the eighth or ninth week, when the increase of these two elements lags behind. There was a decrease in calcium and magnesium in both the stems and the leaves at the time the heads were forming. This last result corroborated the findings of Hornberger (12). Russel (20) notes that magnesium, like phosphorus, finally moves to the seed, and is thus in contrast with calcium and potassium which remain behind in the leaf and straw. According to Hoagland (11) marked absorption of all the nutrient elements in sand and water cultures took place at all periods up to

the final stage of growth. Whiting and Richmond (22) report that the calcium and magnesium content of the tops of the sweet clover plant changed but little during the first season's growth and that there was a ratio of about 3:1 for these elements. However, during the second season's growth the calcium and magnesium content decreased noticeably, there being one-third as much present at the end of the period of growth as at the beginning.

Widstoe (23) gave data to show the proportions of stem to leaves throughout the growing period of alfalfa. In the beginning there was a greater proportion of stems than of leaves—the amounts being 71.48 per cent stems and 28.52 per cent leaves. At maturity the proportion had changed to 35.83 per cent stems, 57.23 per cent leaves, and 6.94 per cent blooms. In the second cutting the proportions at the time buds were beginning to form was 51.03 per cent stems and 48.97 per cent leaves, and at maturity 30.51 per cent stems and 67.02 per cent leaves. Widstoe decided that alfalfa did not start to bloom until the leaves and stems were present in nearly like proportions, varying between 50 to 60 per cent of stalks and 40 to 50 per cent of leaves.

PLAN OF EXPERIMENT

The fundamental object of the work undertaken here was to determine what variations may occur in the composition of alfalfa plants grown on different soil types. The deep rooting habit of the alfalfa plant encourages the expectation that soil types do not greatly influence the composition of the plants. Millar (18) showed that the roots of alfalfa are able to obtain food from the substrata if the materials there are available. If alfalfa plants are able to obtain the mineral elements from any portion of the soil profile where the supply is abundant, there should be little variation in the composition of plants grown on different soil types because of the feeding range.

The results here reported are based on studies which were conducted during the growing season of 1928, beginning as soon as alfalfa had attained sufficient size for sampling. The first samples were taken on May 8 and thereafter at two-week intervals until June 7. After this date samples were collected when the plants had reached fall bloom, the date of which varied considerably on the different soil types. The samples taken on June 7 were just beginning to show buds. Three samples were obtained from the second growth of alfalfa, the first being taken on July 24 and the last at the full bloom stage. Thus the data obtained would not only show variations due to soil type, but would also give the variations at different stages of growth. Separate analyses were made of the leaves and stems and of the expressed juice of leaves and stems.

Alfalfa growing on the following soil types was used: Coloma loamy sand, Plainfield loamy sand, Hillsdale sandy loam, Fox sandy loam, Conover loam, Brookston loam (heavy), and Miami silt loam.

The alfalfa used in this study was of the Grimm variety, all the fields being located in Ingham County, five of them within 12 miles of East Lansing and

the remaining two, on Brookston and Fox soil, at distances of 17 and 25 miles respectively.

EXPERIMENTAL PROCEDURE

All alfalfa samples were gathered between 8 and 10 a.m. on clear days, as McCool and Millar (17) had found that the freezing point lowerings indicated variations in the plant material at different hours of the day. The samples were placed immediately in a saturated atmosphere and brought to the laboratory in this condition.

As soon as possible after getting the samples to the laboratory, the leaves and stems were separated and 10-gm. samples of each were weighed out for total analysis and moisture determination. The remaining material was pressed to furnish the juice for analysis.

The juice was expressed from all samples at seven tons pressure per square inch, a constant pressure being necessary, as previous workers (8, 10, 13, 17) have shown that the concentration of the expressed juice varies according to the pressure applied and the previous treatment of the plant material. The specific gravity of the juice was determined and portions of about 10 gm. were then weighed out into porcelain crucibles for ashing. The juice thus obtained, along with the plant material weighed out as stated before, was ashed in a muffle furnace at dull red heat, taken up in five normal hydrochloric acid, and made up to 100 cc. The calcium determinations were made by the usual official volumetric method, 25-cc. aliquots being used for each determination. Twentieth normal $KMnO_4$ was used to permit greater accuracy, and all results reported are averages of closely agreeing duplicates. The magnesium determinations were made by the volumetric method of Handy (9); the filtrates from the calcium determinations being carefully obtained for this purpose. Twentieth normal KOH was used as the alkali and the averages of closely agreeing duplicates only are reported in this paper. No difficulties were experienced in these determinations as long as the described methods were adhered to and great care was observed in washing and drying filtrates.

EXPERIMENTAL RESULTS

Calcium content of first growth alfalfa stems and leaves

Calcium is such an important element in animal metabolism and its deficiency in a ration is followed by such objectionable effects that some knowledge of the variations which may occur in the calcium content of alfalfa is desirable, especially in the compounding of rations for poultry and swine.

The calcium content of alfalfa stems grown on different soil types and analyzed at different growth stages is given in table 1. According to these data there was a noticeable and consistent variation in the calcium content of the alfalfa grown on the different soil types throughout the growth period. More calcium was found in the stems of plants taken from the heavy soil

types than from the lighter types at each stage of growth except that of full bloom, July 2, where the calcium content dropped on Brookston and Miami soils. The loam soils, Hillsdale and Fox, gave stems with the highest calcium content throughout the period of growth.

Generally a higher percentage of calcium was present in the stems at maturity than at the early stage of growth and the increase was quite uniform on all the types. With the exception of the stems of the plants grown on Coloma loamy sand, a decrease in the calcium content occurred after the plants reached the budding stage, or on June 7, and this decrease was greater in the stems obtained from the very heavy soils.

The calcium content of alfalfa leaves as percentage of dry material is also given in table 1. Much greater variation was evident in the amounts of calcium

TABLE 1

Calcium content of first growth alfalfa stems and leaves at different stages of growth when grown on different soil types*

SOIL TYPE	STAGES OF GROWTH							
	Very young May 8		May 22		June 7		July 2	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	0.770	1.103	0.777	0.826	0.782	2.140	0.767	2.522
Coloma	0.897	1.211	0.842	1.683	0.885	1.695	0.913	2.930
Hillsdale	0.850	1.182	1.051	2.120	1.025	2.339	0.911	4.060
Fox	0.956	1.571	0.993	1.737	1.172	3.108	1.167	4.020
Conover	0.949	1.353	.....	1.710	1.404	3.830	Not obtained	
Brookston	0.946	1.161	1.283	1.748	1.183	2.568	0.864	2.250
Miami	0.915	1.468	1.070	1.917	1.561	2.148	0.972	2.610

* Dry basis.

present in the leaves of alfalfa grown on different soil types than was found for alfalfa stems.

Throughout the period of growth, the calcium content was highest in the leaves grown on the soils of medium texture as compared with the very light or the very heavy soils.

The calcium content of alfalfa leaves increased uniformly as the plants became older and the percentage of calcium at full bloom was from two to nearly four times as great as in the very young leaves. The leaves grown on Brookston loam soil were the only ones to show a decrease in calcium after the budding stage, and the increase in the leaves obtained from Miami soil was small. These facts, and those noted in the case of the stems where the decrease after the budding stage was greater on these two heavy soils, may be explained by the fact that in these plants there was an adjustment made between the calcium present and some other cation as potassium, which did not occur in the plants obtained from the lighter soils.

The calcium content of alfalfa leaves was greater than that found for stems of the same plants. In the very young plants, this difference in calcium content of the leaves and stems was slight, but at maturity it was much greater, there being nearly four times as much calcium in the leaves as in the stems.

The data on the percentage of calcium present in the expressed juice of alfalfa stems grown on different soil types are given in table 2. It is apparent that there were differences in the calcium content of the juice obtained from stems of plants grown on the different soil types, although they are not consistent throughout the growing period. Generally the calcium content was low in the juice of stems grown on the very sandy soils and high on the sandy loams and the loams.

TABLE 2

Calcium content of expressed juice of first growth alfalfa stems and leaves at different stages of growth on different soil types

SOIL TYPE	STAGE OF GROWTH							
	May 8		May 22		June 7		July 2	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	0.133	0.175	0.060	0.185	.....	0.332	0.074	0.389
Coloma	0.133	0.275	0.068	0.227	0.095	0.262	0.145	0.618
Hillsdale	0.086	0.247	0.088	0.287	0.106	0.415	0.177	0.754
Fox	0.111	0.219	0.092	0.316	0.144	0.564	0.154	0.903
Conover	0.123	0.237	0.082	0.240	0.193	0.623	Not obtained	
Brookston	0.147	0.244	0.133	0.297	0.167	0.439	0.125	0.408
Miami	0.092	0.210	0.104	0.255	0.165	0.502	0.196	0.713

Although no uniform increase occurred in the percentage of calcium in the juice of alfalfa stems, there was a general tendency for the concentration to be higher in the juice of the mature stems than in that of the young stems. The plants obtained from Plainfield and Brookston soils were the only exceptions. The plants obtained from Brookston soil likewise were the only ones which showed a depression of calcium in the expressed juice of the stems after the budding stage, due again perhaps to the balance between the cations noted before. The period of greatest depression occurred on all of the soil types, with the exception of Hillsdale and Miami soils, at the time the second sampling was made (May 22). Although these exceptions cannot be explained, this depression at this time was probably due to a greater moisture content in the stems, a fact which will be brought out in subsequent data.

The calcium content of the expressed juice of alfalfa leaves grown on different soil types is given in table 2, along with the similar data relative to the juice

of the stems. Large differences existed in the concentration of calcium in the juice obtained from leaves grown on different soil types, although the differences were not especially great in the juice of the young leaves.

The greatest concentrations of calcium appear to have been present in the juice of leaves grown on the heavy soil types, although that of the juice of leaves grown on Brookston loam was quite low. As usual, greater percentages of calcium were present in the juice of the leaves grown on Fox and Conover sandy loams than on either the very light or the very heavy soils.

A rather uniform increase in calcium content occurred in the juice of leaves as the plants became older and there does not appear to have been any stage of growth where a general depression occurred. However, the increase in concentration was small on May 22 on nearly all of the soil types and on some there was a depression of calcium in the juice of the leaves at this time. These

TABLE 3

Magnesium content of first growth alfalfa stems and leaves at different stages of growth on different soil types*

SOIL TYPE	STAGE OF GROWTH							
	May 8		May 22		June 27		July 2	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	0.247	0.256	0.313	0.285	0.184	0.258	0.156	0.337
Coloma	0.268	0.224	0.256	0.309	0.228	0.199	0.220	0.321
Hillsdale	0.231	0.226	0.400	0.244	0.240	0.241	0.238	0.330
Fox	0.396	0.268	0.366	0.297	0.369	0.397	0.325	0.587
Conover	0.414	0.313	0.495	0.250	0.484	0.444	Not obtained	
Brookston	0.319	0.241	0.388	0.203	0.325	0.263	0.174	0.263
Miami	0.336	0.265	0.346	0.284	0.432	0.262	0.211	0.298

* Dry basis.

conditions were probably due to the greater moisture content of the leaves at this stage of growth. Without exception, the concentration of calcium was greater in the juice of mature leaves than in that of the young leaves.

The percentage of calcium in the expressed juice of alfalfa leaves was higher than that of the juice of alfalfa stems. In the young plants, the concentration of calcium in the juice of leaves was about twice as great as in the juice of stems, but as the plants became older the difference became greater until at maturity the concentration of calcium in the juice of the leaves was five or six times that in the juice of the stems.

About one-fifth as great a percentage of calcium was present throughout the period of growth in the juice of alfalfa leaves as in the tissue of leaves. The increase in concentration of calcium in the juice was accompanied by a similar increase of calcium in the tissue of the leaves.

Magnesium content of first growth alfalfa stems and leaves

Although probably of less importance than calcium from the nutritional standpoint, magnesium is an important constituent of all plant materials and a knowledge of variations in the amounts present in plants at different times in the growth period and as affected by soil type is undoubtedly valuable.

The magnesium content in percentage of dry material present in the stems of first growth alfalfa on different soil types is given in table 3. It is evident that throughout the growth period there was considerable difference in the amount of magnesium present in the stems grown on the various types of soil. During the entire growth period the heavier soil types gave stems containing noticeably greater amounts of magnesium than did the light soils, although as the plants became older this influence of soil texture became less marked. Higher magnesium content was always evident in the stems grown on the sandy loam soils, Fox and Conover, than in the stems grown on either the very heavy or the very light soils.

A depression of greater or less magnitude occurred in the magnesium content of stems grown on all of the soil types after the budding stage. On some of the soil types, as Coloma, Plainfield, and Brookston, the decrease of magnesium in the stems may be almost uniform from the early stage of growth to maturity. With but one exception the percentage of magnesium was lower in the mature stems than in the very young stems samples on May 8.

The magnesium content of alfalfa stems was much lower than the calcium content throughout the period of growth. Although the amount of magnesium varied very uniformly with that of calcium, it did not parallel the amount of the calcium, at times rising and falling independently of it.

Table 3 likewise contains data showing the magnesium content of first crop alfalfa leaves at several stages of growth on different soil types. Variations appeared in the percentages of magnesium in the leaves grown on the different soil types, but until the plants approached maturity the differences were not great. At no time during the period of growth could the influence of soil texture be discerned except insofar as the leaves from plants grown on Fox and Conover sandy loams were generally somewhat higher than those produced on the other soil types. Usually the magnesium content was as high in the leaves grown on sandy soils as that of the leaves obtained from heavy soils.

The amount of magnesium increased in the leaves of alfalfa on all the types as the period of growth advanced. This increase was more evident in the leaves grown on loamy sands and sandy loams than in those grown on the heavier loam soils, and on only one type was this increase uniform from first to last. Thus periods of depression were noted on all soil types except Fox sandy loam at one or another of the periods of sampling.

The magnesium content of alfalfa leaves was about equal to that of alfalfa stems at each stage of growth on all the soil types. A deviation from this

occurred at the mature stage when the percentage of magnesium in the leaves became greater than that in the stems, partly by because of the increase in the leaves after the budding stage and partly because of the decrease or depression noted in the stems at the same time.

The magnesium content of alfalfa leaves was always much lower than the calcium content. The proportion of magnesium to calcium was about 1:5 in the very young plants and increased to about 1:8 at maturity. This increase in the magnesium-calcium ratio was not noted for stems; the ratio remaining about 1:4 throughout the entire growth period.

Table 4 shows the percentages of magnesium present in the expressed juice of first growth alfalfa stems grown on different soil types. The influence of soil type was evident throughout the growing period of the plant, but at no stage of growth was there any evidence that the texture of the soil was related

TABLE 4

Magnesium content of expressed juice of first growth alfalfa stems and leaves at different stages of growth on different soil types

SOIL TYPE	STAGE OF GROWTH							
	May 8		May 22		June 7		July 2	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	0.084	0.075	0.038	0.038	0.026	0.052	0.045	0.076
Coloma	0.053	0.079	0.038	0.043	0.036	.....	0.068	0.096
Hillsdale	0.038	0.056	0.045	0.052	0.050	.....	0.067	0.088
Fox	0.047	0.060	0.052	0.064	0.048	0.072	0.081	0.115
Conover	0.034	0.053	0.055	0.064	0.076	0.076	.....	.....
Brookston	0.064	0.078	0.060	0.052	0.060	0.062	0.052	0.067
Miami	0.041	0.051	0.052	0.050	0.062	0.062	0.084	0.088

to the concentration of magnesium in the juice of the stems except the fact that at the two intermediate growth stages the concentration was least in the stems grown on Plainfield and Coloma loamy sands.

Except on Plainfield loamy sand and Brookston loam, the concentration of magnesium in the juice of alfalfa stems was greater at maturity than at the early part of the growth period. On some of the types, as Hillsdale sandy loam, Conover sandy loam, and Miami silt loam, the increase in the concentration of the juice of stems was uniform from first to last; on the other types there was a depression in the amount of magnesium present in the juice of the stems at one of the stages of growth succeeding the first.

There was a smaller amount of magnesium in the juice of alfalfa stems than there was calcium and the ratio remained about 1:2 or 1:3 throughout the period of growth. The soil type which gave a high calcium content in the juice of stems likewise gave a high magnesium content, and the fluctuations

during the growing period occurred somewhat nearly at the same time for both calcium and magnesium.

The magnesium content of the juice of alfalfa leaves grown on the different soils is also given in table 4. That variations in the concentration of the juice of leaves grown on different soil types occurred, is quite evident and they continued throughout the growth of the plant. In the very young plants there appeared to be no influence of soil texture but as the plants became older those grown on the heavy soils, and especially those grown on the sandy loams, showed a higher magnesium content in the juice of their leaves than did those grown on the light sandy soils. At the stage of full bloom little effect of soil texture on the magnesium content of the juice was evident. At this time Fox sandy loam gave leaves with a considerably greater amount of magnesium in

TABLE 5

Calcium content of second growth alfalfa stems and leaves at different stage of growth on different soil types*

SOIL TYPE	STAGE OF GROWTH					
	July 24		August 7		Full bloom	
	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	1.198	1.289	1.261	1.598	.....	.....
Coloma	1.170	.....	1.044	1.895	1.092	3.690
Hillsdale	1.125	1.618	1.038	2.080	1.060	2.19†
Fox	2.047	1.787	1.047	2.065	0.932	2.550
Conover	1.410	2.020	1.249	2.388	0.900	3.955
Brookston	1.337	1.874	1.158	3.432	0.991	2.680
Miami	1.347	1.792	.....	.....	0.864	2.950

* Dry basis.
† Half bloom.

their juice than did any others, and the sandy soils were about equal to the heavy soils in this respect.

Greater amounts of magnesium were present in the juice of mature alfalfa leaves than in immature ones, though the difference was very small. On all of the soil types studied, except the sandy loams, there was a depression in the concentration of magnesium in the juice of alfalfa leaves on May 22. This depression, which corresponds to the depression noted for the calcium content at this stage of growth, was most noticeable on the sandy soils.

The magnesium content of the juice of alfalfa leaves was but slightly higher at any time than that of the stem juice. The relative concentrations remained about the same during the growing period so that at no time was the concentration of the juice of one much different from that of the other.

Much smaller amounts of magnesium than of calcium were present in the juice of first growth alfalfa leaves. The proportion of magnesium to calcium

was less in the young plants, about 1:4, but increased as the plants become older until it was about 1:7, or even wider.

Calcium and magnesium content of second growth alfalfa stems and leaves and in their expressed juices

The analysis of second growth alfalfa showed that the calcium and magnesium contents of the tissue and of the juice of both stems and leaves corresponded quite closely to those found in the first crop. Some disagreements were found, however, which are worthy of note.

More calcium was present throughout the growth period, and a more rapid and more general decrease occurred in the percentage of calcium, as the period of growth advanced, in the second crop stems (table 5) than in the first crop stems.

TABLE 6

Calcium content of expressed juice of second growth alfalfa stems and leaves at different stages of growth on different soil types

SOIL TYPE	STAGE OF GROWTH					
	July 24		August 7		Full bloom	
	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	0.104	0.204	0.075	0.198	.....	.....
Coloma	0.122	0.174	0.084	0.243	0.202	0.892
Hillsdale	0.106	0.200	0.094	0.280	0.108	0.364*
Fox	0.106	0.247	0.112	0.345	0.168	0.668
Conover	0.134	0.295	0.127	0.403	.....	.....
Brookston	0.128	0.290	0.130	0.430	0.170	0.678
Miami	0.115	0.245	.....	.....	0.157	0.708

* Half bloom.

Greater amounts of calcium appeared in the leaves of the second crop (table 5) when the plants were young than occurred in those of the first crop, but the increase of calcium from early growth to maturity was slower in the second growth leaves, resulting in a lower content at full bloom than was observed in the first crop leaves. The least increase of calcium with advancing age occurred in the leaves of plants obtained from the soil types which had given up the greatest amounts of calcium to the first crop.

From table 6 it is evident that similar amounts of calcium were present in the expressed juice of both stems and leaves of the two crops throughout the growing period.

Soil type produced variations in the calcium content of the second crop which were similar in magnitude to those produced in the first crop but the influence of soil texture was much less evident and became practically negligible

toward the close of the growth period, perhaps because of an equalizing of the supply of calcium in the nutrient medium.

Tables 7 and 8 show that only the following differences occurred in the magnesium content of the second crop in comparison with that of the first crop of alfalfa.

TABLE 7

Magnesium content of second growth alfalfa stems and leaves at different stages of growth on different soil types*

SOIL TYPES (INCREASING TEXTURES)	DATE OF SAMPLING					
	July 24		August 7		Full bloom	
	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	.....	0.446	0.300	0.374	.....	.....
Coloma	0.690	.....	0.322	0.311	0.408	0.489
Hillsdale	0.578	0.583	0.292	0.490	0.348	0.393†
Fox	0.648	0.535	0.184	0.310	0.310	0.366
Conover	0.760	0.539	0.537	0.502	0.501	0.741
Brookston	0.537	0.523	0.351	0.381	0.246	0.381
Miami	0.798	0.470	.....	.....	0.360	0.489

* Dry basis.
† Half bloom.

TABLE 8

Magnesium content of expressed juice of second growth alfalfa stems and leaves at different stages of growth on different soil types

SOIL TYPES (INCREASING TEXTURES)	DATE OF SAMPLING					
	July 24		August 7		Full bloom	
	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	0.093	0.088	0.048	0.072	.....	.....
Coloma	0.079	0.081	0.045	0.064	0.129	0.134
Hillsdale	0.088	0.088	0.069	0.088	0.076	0.086*
Fox	0.084	0.096	0.048	0.074	0.084	0.122
Conover	0.112	0.105	0.105	0.093	.....	.....
Brookston	0.096	0.091	0.086	0.098	0.103	0.110
Miami	0.100	0.084	.....	.....	.....	0.144

* Half bloom.

Greater amounts of magnesium were present in the tissue and in the juice of both stems and leaves of the second growth than occurred in the first growth.

A more rapid decrease took place in the magnesium content of the tissue of second growth stems than in that of the first growth stems as the period of

growth advanced. In the juice of the stems of the second crop a smaller increase of concentration of magnesium occurred than was found in the juice of the first growth stems.

A tendency was apparent for the magnesium content of the tissue of second crop leaves to decrease with advancing age in contrast to the general tendency for it to increase in the tissue of first growth leaves.

Soil texture was less marked in its influence upon the magnesium content of the second crop of alfalfa than upon that of the first crop. In the second growth the Fox soil appeared to have lost its ability to produce alfalfa containing the large amounts of calcium and magnesium which were observed in the plants obtained from that soil during the first growth period.

Calcium and magnesium content of the plant tissue of alfalfa stems and leaves free of all sap

The quantity of any substance found in moisture-free plant tissue consists of the amount of this substance present in the woody tissue plus the amount originally present in the juice of the plant and left in the tissue upon drying. In order to determine the amount of any substance which makes up a part of the structural tissue of the plant it is necessary to separate from this the amount contained in the juice of the green plant material.

Attempts have been made by some workers (5, 20) to extract separately material of the vacuole portion of plant cells and the material contained in the protoplasm. The methods used were chemical, as the application of extreme mechanical methods was considered to break open the cell wall and allow the escape of some of the protoplasmic material.

In the work presented here the woody plant tissue is considered to consist of cell walls only. Any material in solution or suspension in the vacuole or protoplasmic material is not considered as incorporated in the structural tissue of the plant. Because of this, the total material expressed under rather high pressure, as seven tons per square inch, is assumed to represent the plant substance other than that of the woody tissue, although there is considerable controversy on this point among a number of workers (8, 9, 12).

If the amount of a substance present in the green material of the plant and the amount in the liquid portion of the plant material (represented here by the content in the expressed juice of the plant) are known, the amount of the substance in the woody tissue can be found as the difference of these two. This procedure is represented by the following equation for the determination of a substance *A*:

$$A \text{ in woody tissue} = \text{per cent } A \text{ in green plant material} - \text{per cent } A \text{ in juice} \times \text{grams juice per grams green material.}$$

The data necessary in the use of this equation consist of the percentage of the substance sought in the green tissue of the plant, the percentage of the substance in the expressed juice of the green material, and the exact moisture

content of the green material. The moisture content of the plant equals, with but slight error, the total amount of plant juice which could be extracted if it were possible to press the material so greatly that it would be moisture free.

Results obtained from the use of the above formula are incorporated in tables 9 to 12 inclusive. The moisture content of the materials is presented subsequently in table 13.

The amounts of calcium in the woody tissue of first growth alfalfa stems and leaves when the plants were very young and also when they were at full bloom are given in table 9. A much smaller amount of calcium was present in the woody tissue of young alfalfa stems than in that of old ones. This indicates that during the period of growth a certain amount of calcium was being added to the tissue. The amounts of calcium deposited in the tissue of the stems after the plants were sampled on May 8 until they were in full

TABLE 9

Relative amounts of calcium present in the woody tissue of first growth alfalfa stems and leaves in early growth and at maturity

When grown on different soil types

SOIL TYPE	EARLY STAGE, MAY 8				MATURE STAGE					
	Mgm. Ca per gm. green material		Mgm. Ca in tissue of 1 gm. green material		Mgm. in gm. green material		Mgm. Ca in tissue of 1 gm. green material		Amount added after May 8, mgm. per gm.	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
Plainfield	1.14	2.06	0.003	0.634	2.00	5.78	1.50	0.297	1.497	−0.337
Coloma	1.60	2.75	0.500	0.619	2.40	6.97	1.33	0.150	0.830	−0.469
Hillsdale	1.38	2.50	0.664	0.551	2.38	9.11	1.31	0.327	0.646	−0.224
Fox	1.56	3.47	0.627	0.177	2.56	9.76	1.21	0.294	0.583	0.117
Brookston	1.84	2.64	0.655	0.075	2.00	6.00	1.03	0.300	0.375	0.225
Miami	1.565	3.24	0.796	0.161	2.64	7.08	1.21	0.190	0.414	0.030

bloom varies from 0.375 mgm. per gram of green material to 1.49 mgm. per gram.

Widely different amounts of calcium were present in the woody tissue of first growth alfalfa stems grown on the different soil types. Stems grown on Plainfield soil contained in their woody tissue when very young, only 0.003 mgm. of calcium per gram, whereas those grown on Miami soil contained in their woody tissue 0.796 mgm. per gram. At maturity the stems grown on Plainfield soil contained 1.50 mgm. calcium per gram in their woody tissue and those grown on Brookston soil contained only 1.03 mgm. per gram. Consequently it appears that the texture of the soil influenced the amount of calcium deposited in the woody tissue, that of stems grown on heavy soil types containing more calcium when very young than that of stems grown on light soils, and the woody tissue of stems grown on heavy soils containing less at full bloom than that of stems grown on light soils.

There was a much greater amount of calcium deposited in the woody tissue of stems grown on the sandy soils during the period of growth following the first sampling. These data indicate that at no time during the period of growth was calcium actually removed from the woody tissue of the stems, as may have been interpreted from the analysis of the dry material (table 1).

The data in table 9 show that noticeable differences existed in the amounts of calcium deposited in the woody tissue of the first growth leaves from the different soil types both in the early stage of growth and at full bloom. Greater amounts of calcium were present in the woody tissue of the leaves grown on heavy soils when the leaves were young. As the period of growth advanced, however, calcium was removed from the woody tissue of leaves grown on the ligher soils whereas calcium was deposited in the tissue of leaves grown on heavier soils, with the result that the amount of calcium in the woody tissue of

TABLE 10

The relative amounts of magnesium in the woody tissue of first growth alfalfa stems and leaves at early growth and at maturity

When grown on different soil types

SOIL TYPE	EARLY STAGE, MAY 8				MATURE STAGE					
	Mgm. Mg per gm. green material		Mgm. in tissue of 1 gm. green material		Mgm. Mg per gm. green material		Mgm. Mg. in tissue of 1 gm. green material		Amount Mg added after May 8	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
Plainfield	.....	0.48	.....	....	.....	0.77	.....	0.16	.....	....
Coloma	0.480	0.50	0.040	....	0.476	0.77	0.073	0.04	0.033	....
Hillsdale	0.384	0.48	0.066	0.03	0.624	0.74	0.128	0.06	0.062	0.03
Fox	0.648	0.60	0.249	0.13	0.720	1.44	0.085	0.58	0.164	0.45
Brookston	0.624	0.55	0.106	....	0.408	0.69	0.003	0.18	0.103	....
Miami	0.576	0.60	0.236	0.21	0.576	0.81	0.117	0.17	0.119	−0.04

leaves grown on ligher soils was less at full bloom than in that of leaves grown on the heavier soils, except in the case of Miami soil.

Smaller amounts of calcium were present in the woody tissue of alfalfa leaves of the first crop at both stages of growth studied than was the case with the stems. Also less calcium was deposited in the woody tissue of the leaves than of the stems during the period of growth. Apparently there was a translocation of calcium from the leaves of alfalfa grown on the light sandy soils.

In table 10 are given the relative amounts of magnesium in the woody tissue of the stems and of the leaves of first growth alfalfa at early growth and at full bloom. Much smaller amounts of magnesium than of calcium were present in the woody tissue of stems at any time, and it appears that at least in stems grown on heavy soil types there was a removal of a portion of the magnesium as the plants advanced in age.

The amount of magnesium in the woody tissue of the alfalfa leaves was

usually about equal to the amount of calcium present, and in some cases the amount was even greater. Although the data are incomplete it appears that only small amounts of magnesium were deposited in the woody tissue of leaves in the latter portion of the growth period and some may even have been

TABLE 11

Relative amounts of calcium in the woody tissue of second growth alfalfa stems and leaves in early growth and in full bloom

When grown on different soil types

SOIL TYPES	EARLY STAGE				FULL BLOOM					
	Mgm. Ca in gm. green material		Mgm. Ca in actual tissue		Mgm. Ca in gm. green material		Mgm. Ca in actual tissue		Amount Ca added after July 24	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
Plainfield	1.90	3.03	1.03	1.49	....	....	....	....	....	....
Coloma	1.38	....	0.21	....	3.34	7.38	1.94	1.06	1.73	....
Hillsdale	1.58	2.95	0.66	1.32	....	....	....	....	....	....
Fox	1.83	3.52	0.92	1.54	3.02	7.94	1.88	3.35	0.96	....
Conover	1.95	4.10	0.80	1.76	....	....	....	....	....	....
Brookston	2.26	4.08	1.21	1.82	3.18	7.58	2.02	2.73	0.82	0.92
Miami	1.88	3.60	0.89	1.65	2.79	8.26	1.67	3.17	0.78	1.52

TABLE 12

Relative amounts of magnesium in the woody tissue of second growth alfalfa stems and leaves in early growth and in full bloom

When grown on different soil types

SOIL TYPE	EARLY GROWTH				FULL BLOOM					
	Mgm. Mg in gm. green material		Mgm. Mg in tissue of 1 gm. green material		Mgm. Mg. in 1 gm. green material		Mgm. Mg in tissue of 1 gm. green material		Mgm. Mg added after July 24	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
Plainfield	....	1.05	....	0.38	....	....	....	....	....	....
Coloma	0.81	....	0.04	....	1.83	1.68	0.94	0.72	0.90	....
Hillsdale	0.81	1.01	0.05	0.29	....	....	....	....	....	....
Fox	0.91	1.05	0.19	0.28	1.94	1.86	1.37	1.92	1.18	1.64
Conover	1.05	1.08	0.08	0.25	....	....	....	....	....	....
Brookston	0.91	1.03	0.13	0.32	1.92	1.69	1.23	0.90	1.10	0.58
Miami	1.15	0.96	0.29	0.30	1.89	1.37	1.05	0.34	0.76	0.04

removed. Apparently soil texture influenced the amount of magnesium deposited in the tissue at the early stage of growth, when there was more in the stems and leaves grown on heavy soil types.

Data showing the amounts of calcium in the woody tissue of second growth alfalfa stems and leaves are given in table 11. In the case of stems, soil texture did not appear to influence the amount of calcium in the woody tissue but it

did influence the amounts that were added during the final stages of growth, greater amounts being added to the stems grown on light soil types.

About equal amounts of calcium were present in the stems of first and second growth alfalfa when very young, but in the more mature plants the amount became greater in the second crop stems, and greater amounts were added during the growth period. Noticeably greater amounts of calcium were present in the woody tissue of second crop leaves during the entire growth period, and greater amounts were deposited in the tissue during the life of the plant.

TABLE 13

Moisture content of first and second growth alfalfa stems at different stages of growth and on different soil types

Per cent on dry basis

DATE OF SAMPLINGS	SOIL TYPE						
	Coloma	Plainfield	Hillsdale	Fox	Conover	Brookston	Miami
First growth							
May 8	82.10	85.35	83.85	83.73	83.75	80.55	82.90
May 22	86.95	97.75	86.35	86.70	85.95	83.95	86.15
June 7	83.20	81.85	84.00	81.80	79.85	79.70	82.25
July 2	73.90	73.85	73.85	77.90	.....	76.85	72.80
Second growth							
July 24	88.20	84.15	86.90	86.05	86.10	82.05	86.05
August 7	82.90	84.80	77.85	75.25	77.25	74.75	.....
Full bloom	69.40	.....	.....	67.60	.....	67.90	68.37

Greater amounts of calcium were present in the woody tissue of second growth alfalfa leaves than in that of the stems. This is the reverse of what was found for alfalfa of the first crop.

Table 12 includes data showing the amounts of magnesium in the woody tissue of second growth alfalfa stems and leaves. Greater amounts of magnesium were present in the leaves of the young plants, but more was deposited in the tissue of the stems as the plants became older, resulting in a greater quantity in the woody tissue of the stems at full bloom. Magnesium was added to the woody tissue of both stems and leaves in every instance.

More magnesium was found in the woody tissue of the stems of the second crop at full bloom than in that of the first crop stems. In the young plants, however, there was little difference in the amounts present in the two crops and a remarkable agreement is evident in the data of tables 10 and 12 relative to this early growth.

Greater amounts of magnesium were present in the woody tissue of second growth leaves than in that of first growth leaves throughout the growth period, and more magnesium was deposited in second crop leaves as the plants became older.

Smaller amounts of magnesium and of calcium were always present in the woody tissue of both stems and leaves of the second crop of alfalfa than in that of the stems and leaves of the first crop.

Relationship of soil type to additional characteristics of the alfalfa plant

Great uniformity is shown in the moisture contents of alfalfa stems and leaves grown on the different soil types by the data presented in table 13.

TABLE 14

Moisture content of first and second growth alfalfa leaves at different stages of growth and on different soil types

Per cent on dry basis

DATE OF SAMPLINGS	SOIL TYPE						
	Coloma	Plainfield	Hillsdale	Fox	Conover	Brookston	Miami
First growth							
May 8	77.03	81.26	78.75	77.62	78.57	77.15	77.75
May 22	79.73	82.30	79.05	79.10	79.60	76.95	76.40
June 7	78.40	81.40	78.15	77.65	77.35	76.25	77.10
July 2	75.95	76.85	77.45	75.45	.....	73.60	72.70
Second growth							
July 24	82.20	76.35	81.70	80.30	79.85	78.20	79.55
August 7	80.75	79.45	78.45	74.95	78.05	75.45	.....
Full bloom	72.00	.....	.....	68.85	.....	71.65	72.05

Although the moisture content of stems and leaves grown on Plainfield soil was usually the greatest and that of stems and leaves grown on Brookston soil was usually lowest, the differences between the two were not great at any stage of growth. Although it is recognized that there were not large differences in the moisture contents of the stems and leaves grown on the several soil types, yet some interesting variations which appear to be related to soil type differences, will be pointed out in the following discussion.

Greater percentages of moisture were present in the stems than in the leaves of alfalfa when the plants were young. The moisture content of both stems and leaves decreased as the plants became older, it being noticeably less at full bloom than in the very young materials. An increase in moisture was noted on almost every soil type for both stems and leaves at the sampling

made on May 22. After this date the decrease in moisture content was quite uniform on all of the soil types.

The moisture content of the stems grown on the different soil types was about equal when the plants were very young; but there was a greater decrease in the amount of moisture in the stems grown on the light soils as the season advanced, resulting in a generally lower moisture content at full bloom in these as compared with the stems grown on the heavy soils.

Contrary to what was found for the stems of alfalfa, the decrease of moisture in the leaves as the growth period advanced was slower on the sandy soils than on the heavy soils. This resulted not only in the fact that the moisture content was higher in the leaves on sandy soils at the full bloom stage but also in the fact that the leaves on sandy soils decreased slowly enough in moisture that they contained more than did the stems at the full bloom stage. This

TABLE 15

Specific gravity of expressed juice of first growth alfalfa stems and leaves at different stages of growth

Specific gravity of distilled water = 1.000

SOIL TYPE	STAGE OF GROWTH					
	May 22*		June 6		July 2	
	Stems	Leaves	Stems	Leaves	Stems	Leaves
Plainfield	1.023	1.035	1.026	1.041	1.031	1.046
Coloma	1.023	1.037	1.029	1.044	1.028	1.054
Hillsdale	1.020	1.041	1.030	1.046	1.032	1.056
Conover	1.026	1.046	1.034	1.054	.....	.....
Fox	1.031	1.046	1.034	1.052	1.032	1.060
Brookston	1.032	1.048	1.040	1.052	1.034	1.054
Miami	1.030	1.047	1.034	1.052	1.038	1.058

* Data not obtained for first sampling.

was not true of leaves grown on the heavy soil types, whose moisture contents were always lower than those of the stems.

Noticeably greater amounts of moisture were present in the second growth alfalfa plants (table 14) than were present in those of the first growth when the plants were very young. But at the full bloom stage the amount of moisture was greater in the first growth alfalfa plants. There appeared to be no relationship between soil type and the moisture content in the second growth alfalfa. Although Brookston stems were still lowest in moisture, Plainfield stems and leaves were no longer highest.

Much greater amounts of moisture were present in the stems of young second growth alfalfa than were present in the leaves. A much greater decrease took place, however, in the moisture content of the stems during the growth period than occurred with the leaves and, as a result, the moisture content at full bloom was greatest in the leaves on all the types studied.

The specific gravity of the expressed juice of first growth alfalfa stems and leaves at different stages of growth, and its relationship to soil types

The specific gravities of the expressed juice of first growth alfalfa stems and leaves at different stages of growth are shown in table 15. Here it is evident that the specific gravity was much greater for the juice of alfalfa leaves than it was for that of stems at all stages of growth.

As the alfalfa plants became older, the specific gravity of the expressed juice of the leaves increased in an almost arithmetical progression on all of the soil types studied. This increase is rapid throughout the period of growth, and agrees rather closely with the increase noted in the calcium and magnesium content of the juice of the leaves.

An increase at early growth was observed in the specific gravity of the juice of alfalfa stems almost identical to that observed for the leaves, but on only two soil types was the increase uniform throughout the growth period. On the remaining soil types the specific gravity either increased very slowly to full bloom or decreased more or less rapidly until it was but slightly higher than that observed in the young plants. Any increase or decrease in the specific gravity of the juice of alfalfa stems did not correspond uniformly to similar increases or decreases in the calcium and magnesium content of the juice of stems as given previously, although on three of the soil types, Plainfield, Miami and Brookston, there was an agreement.

TABLE 16

Specific gravity of expressed juice of second growth alfalfa stems and leaves at different stages of growth and on different soil types

Specific gravity of distilled water = 1.000

SOIL TYPE	STEMS		LEAVES	
	Stage of growth			
	August 7	Maturity	August 7	Maturity
Plainfield	1.026	.....	1.040	.....
Coloma	1.021	1.040	1.036	1.072
Hillsdale	1.028	.....	1.042	.....
Fox	1.028	1.048	1.048	1.082
Conover	1.028	.....	1.045	.....
Brookston	1.030	1.044	1.050	1.068

Marked differences were found in the specific gravities of the juice from stems and leaves of plants grown on the soils of different texture. Table 15 shows that the specific gravity of the juice of both stems and leaves of plants grown on heavy soils was much higher than that of stems and leaves grown on the light soils. This is in general agreement with the data obtained in regard to the calcium and magnesium content of the juice of the stems and leaves, and it would appear, therefore, that these elements were largely responsible for the

specific gravity of the juice. Deviations from this were no doubt due to the presence of other cations which probably varied in amount in the plants grown on the different soil types.

In table 16 is given the specific gravity of the juice of second growth stems and leaves, which was much higher in the full bloom stage than in the case of first crop alfalfa. There was little difference in the values of the two crops when the plants were young, and therefore the increase in specific gravity of the juice as the growth period advanced was much more rapid in second cutting plants. Here, as was found in the first crop, it appears that there was rather a close agreement between the specific gravity of the juice and its calcium and magnesium content, and that these elements were important in determining the specific gravity. This does not lose sight of the fact that other cations were also present, and these may account for the irregularities which were noted.

TABLE 17

Proportion of leaves and stems of alfalfa at budding stage on different soil types

Per cent green material

PART OF PLANT	SOIL TYPE						
	Plainfield	Coloma	Hillsdale	Fox	Conover	Brookston	Miami
Leaves	62.0	59.0	65.4	61.1	59.1	58.0	61.1
Stems	38.0	41.0	34.6	38.9	40.9	42.0	38.9

There appeared to be no relationship between soil texture and the specific gravity of the juice of leaves and stems of second cutting alfalfa.

Proportions of stems and leaves in the alfalfa plant as affected by soil type

Some variations occurred in the proportion of stems and leaves of the alfalfa plants grown on different soil types at the time buds were appearing. Widstoe (23) found that as the period of growth advanced, the percentage of leaves increased. It is noticeable in table 17 that the percentage of leaves was greater on the plants grown on Plainfield, Hillsdale, Fox, and Miami soils, but it is entirely possible that this was due to a more advanced stage of growth on these types; throughout this work, a more luxuriant growth was observed on these soil types and it may be that the budding stage was a few days more advanced than on the other types. Strength is added to this supposition by the fact that the growth was always slowest on Brookston, and here the proportion of leaves was lowest.

Considering these facts, it is probable that there was little difference in the percentages of leaves and stems grown on the different soil types and it is also evident that soil texture did not influence it.

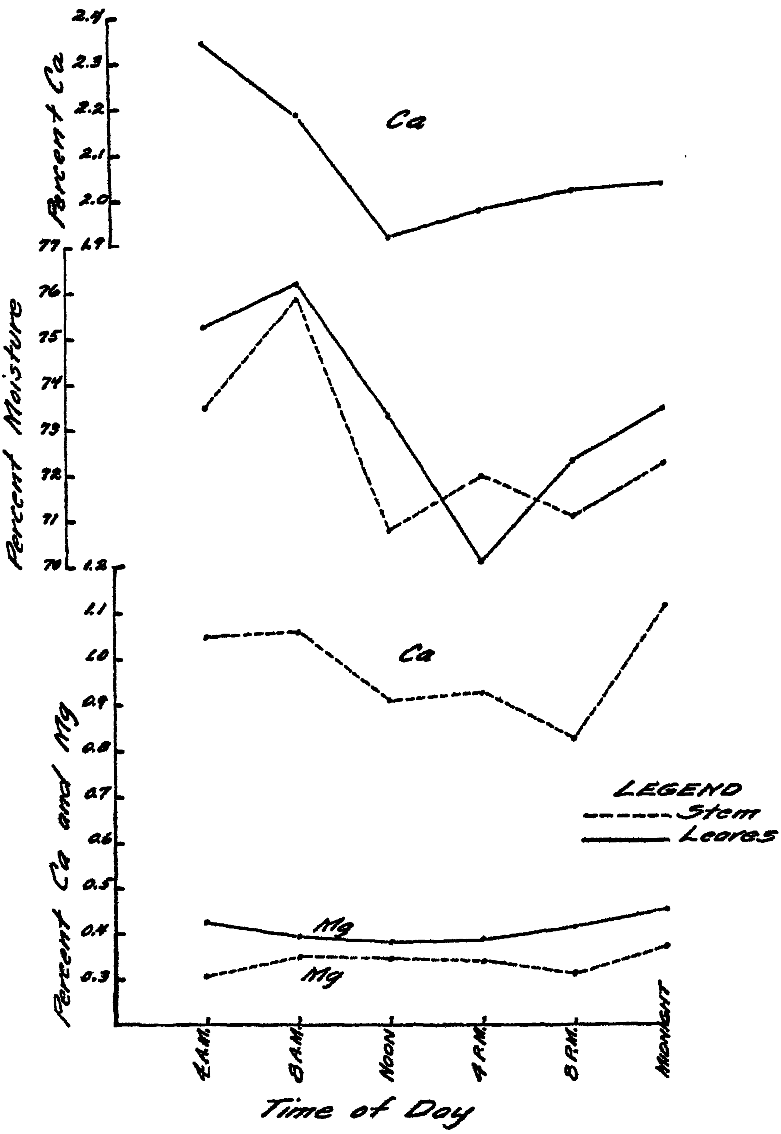


FIG. 1. THE RELATION OF TIME OF DAY TO THE CALCIUM, MAGNESIUM, AND MOISTURE CONTENTS OF ALFALFA STEMS AND LEAVES

Variations in the composition of the alfalfa plant at different hours of the day

Differences in the rate of transpiration and in the physiological activity of the plant occur at different hours of the 24-hour cycle. In order to determine what variations occur in the factors studied in this work, samples of alfalfa grown on Hillsdale sandy loam were taken at 4-hour intervals during the 24-hour day.

That considerable variation occurred in the calcium and magnesium content and in the moisture content of alfalfa stems and leaves at different times of the day is shown by figure 1. These three substances showed a remarkable correlation, a rise or a fall in one being accompanied by almost a proportionate rise or fall in the other two.

In the case of the stems a rather sharp rise in the calcium, magnesium, and moisture content took place in the period of time from 4 until 8 a.m. After 8 o'clock a marked depression occurred and continued until 4 p.m. or even until 8 p.m. During the 4-hour period between 8 p.m. and midnight, there was a sharp rise in the amounts of moisture, calcium, and magnesium present.

The moisture content of the leaves nearly paralleled that of the stems during the 24-hour period, but its relationship to the calcium and magnesium content of the leaves was not so exact as was noted for stems. During the 4-hour period from 4 until 8 a.m. there was an increase in the moisture content but a decrease in the amount of calcium and magnesium. Also during the 4-hour period between noon and 4 p.m. there was a decrease in the moisture content but an increase in the calcium and magnesium content.

At 4 p.m. the lowest moisture content was reached for the leaves and at noon the lowest content of calcium and magnesium was reached. Greater variation in the composition of the leaves took place between 4 a.m. and noon, and the least between noon and 8 p.m.

Variations occurring in the specific gravity and in the calcium and magnesium content of the expressed juice of alfalfa stems and leaves are given in figure 2. For the juice of the stems, the lowest specific gravity occurred at 8 a.m., after which there was a rise until noon and then a constant value maintained until midnight. A decrease in the calcium and magnesium content of the juice also occurred at 8 a.m., followed by a rise lasting until 4 p.m.

Greater variations took place in the specific gravity, the calcium content, and the magnesium content between 8 a.m. and noon than during any other 4-hour period.

Figure 2 also shows variations taking place in the specific gravity and in the calcium and magnesium content of the expressed juice of alfalfa leaves during the 24-hour cycle. Somewhat the same trend was taken by the varying values for the leaves as was observed for the stems, the low points occurring at 8 a.m., followed by increases during the middle of the day.

The greatest variation in specific gravity and in the calcium content occurred from 8 a.m. until noon, followed by a uniform period from noon to

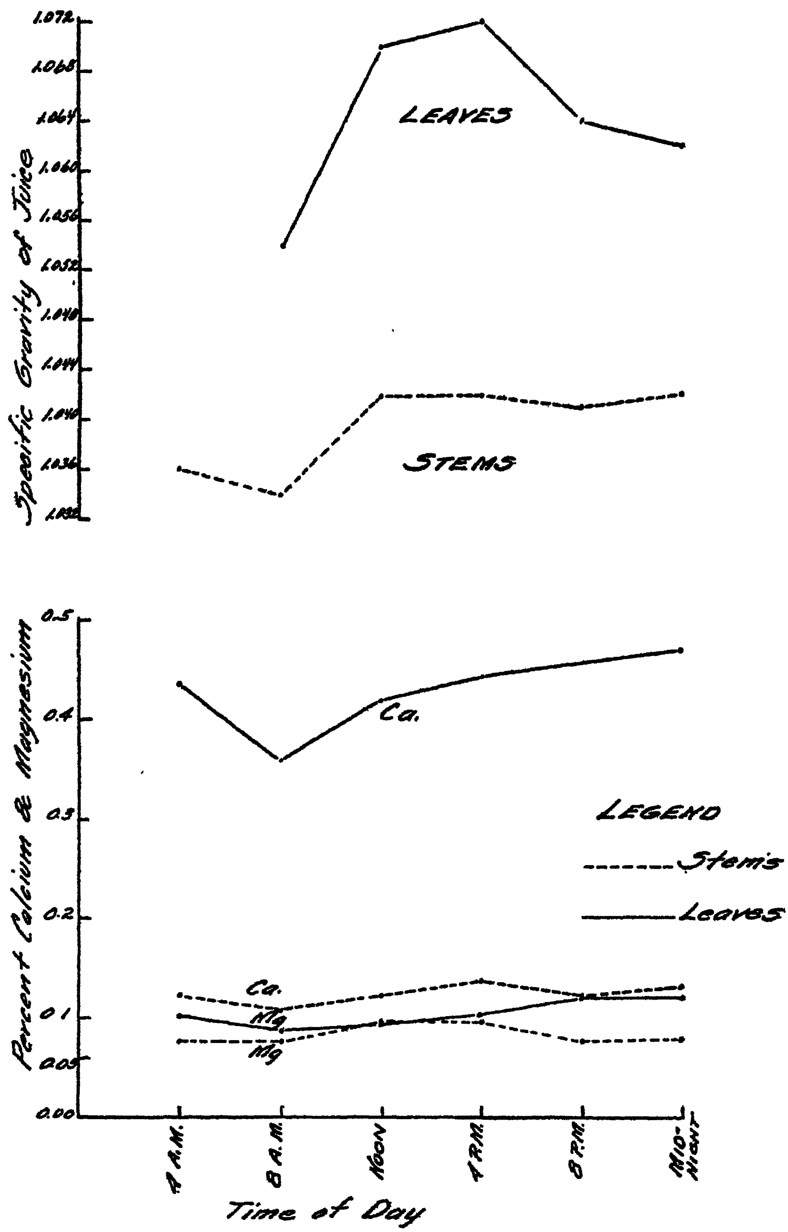


FIG. 2. THE RELATION OF TIME OF DAY TO THE SPECIFIC GRAVITY AND THE CALCIUM AND MAGNESIUM CONTENTS OF THE EXPRESSED JUICE OF ALFALFA STEMS AND LEAVES.

4 p.m. The magnesium content was singularly uniform during the entire day in the expressed juice of alfalfa leaves.

The concentration of calcium and magnesium in the juice of stems and leaves varied inversely as the amounts of these present in the tissue at different times of the day. However, the variations in concentration of the juice were accompanied by like changes in the specific gravity and by unlike changes in the amounts of moisture present in the green material. Since it has been established that a large part of the calcium and magnesium present in the tissue is due to that contained in the juice, a reduction in the juice, even though accompanied by an increased concentration, would bring about a decrease of these elements in the tissue. Evidently this is what occurred to cause a decrease of the two elements in the tissue simultaneously with the decrease in moisture.

SUMMARY AND CONCLUSIONS

Alfalfa stems and leaves and the expressed juice of alfalfa stems and leaves grown on seven soil types were analyzed for calcium and magnesium at different stages of growth. The moisture content and the specific gravity of the expressed juice were determined. The proportion of stems and leaves and the effect of time of day on the composition of the alfalfa plant were determined. In addition, the amounts of calcium and magnesium present in the woody tissue of the leaves and stems free of the amounts in the liquid portion of the plants were determined in very young and in mature plants.

There were marked differences in the calcium and magnesium contents of alfalfa stems and leaves, and of their expressed juice in the plants grown on the different soil types. Likewise marked differences were found in the specific gravity of the juice of stems and leaves and slight differences in the amounts of moisture present in them when obtained from the different soil types.

Generally the calcium content was higher in the dry tissue and in the expressed juice of alfalfa stems and leaves grown on the heavy soil types studied. Sandy loams, however, gave a higher calcium content in these than did the heavier loams.

Soil type had less influence on the second crop than on the first and the effect of soil texture became practically negligible in the second crop. It would appear that with the continued growth of the plants, changes which equalized the facilities with which the plants were able to obtain their supply of calcium and magnesium, had occurred in the soils, resulting in a uniformity of composition (in respect to these elements) of the plants from the different soil types.

No consistent relationship appeared to exist at any time between the texture of the soil and the magnesium content of the alfalfa plant.

More calcium was present in the tissue and in the juice of the alfalfa leaves than in the tissue and in the juice of the stems.

About equal amounts of magnesium were present in the tissue of leaves and stems and also in their juice.

More calcium than magnesium was always present in the tissue and the juice of both the stems and the leaves.

There was either an increase or a decrease in the calcium content of the tissue and of the juice of first growth alfalfa stems as the plants became older, but there was only a decrease in the calcium content of the tissue and juice of second growth stems as the plants advanced in age.

The magnesium content of the tissue of first and second growth alfalfa stems decreased as the plants became more mature.

The calcium content increased in the tissue and in the juice of leaves of the first and second crops of alfalfa as the plants grew older.

With the advance of the growth period, the magnesium content increased in the tissue of first growth alfalfa leaves but decreased in the tissue of the leaves of the second growth.

Greater concentrations of magnesium were usually present in the juice of more mature leaves and stems than in that of the young leaves and stems of both the first and second crops.

The calcium content was higher in the stems and in the young leaves of second growth alfalfa than in the first growth. The content of calcium was higher in mature leaves of first crop alfalfa than in the mature leaves of the second growth.

About equal percentages of magnesium were present in the stems and leaves of first and second growth alfalfa and likewise the concentrations of calcium and magnesium were about equal in the juice of stems and leaves of both cuttings of alfalfa.

Large amounts of calcium were deposited in the woody tissue of alfalfa stems as the plants advanced in age, and greater amounts were added to the stem tissue of the second growth than to that of the first growth.

In the first crop of alfalfa, calcium was added to the woody tissue of the leaves on some of the soil types as the growth period advanced, whereas on other soil types it was removed from the woody tissue. In the second crop calcium was always added to the woody tissue of the leaves.

Greater amounts of calcium were usually present in woody tissue of alfalfa stems than in that of the leaves, and more was deposited in the woody tissue of the stems than in that of the leaves as the period of growth advanced.

Soil type influenced the amount of calcium in the woody tissue of alfalfa stems and leaves. In the early stage of growth the calcium content was low in the woody tissue of stems grown on light soil and low in the tissue of the leaves grown on heavy soils, whereas at maturity the calcium content of the woody tissue of stems was low on the heavy soils and in that of leaves it was low on the light soils.

Much smaller amounts of magnesium than of calcium were present in the woody tissue of first growth alfalfa stems, whereas the amounts of calcium and magnesium were about equal in the woody tissue of leaves.

In second growth alfalfa, greater amounts of calcium than of magnesium were present in the woody tissue of both stems and leaves.

In first growth alfalfa, the amount of magnesium in the woody tissue of stems and leaves was either decreased or increased as the plants became older. In the second crop it always increased in both stems and leaves.

The amount of magnesium in the woody tissue of stems and leaves of both the first and the second crops of alfalfa varied on the different soil types but it did not appear to depend on soil texture.

Greater amounts of magnesium were present in the woody tissue of stems and leaves of second growth alfalfa than in that of the first crop. It appears that the calcium and magnesium present in the plant material were largely due to that contained in the juice of the plant and not incorporated in the tissue itself.

The moisture content of alfalfa stems and leaves decreased as the plants became more mature.

More moisture was present in the stems than in the leaves of alfalfa when the plants were young but it became about equal, or slightly greater in the leaves, as the plants advanced in age, especially in the second crop.

Greater moisture content occurred in the stems and leaves of the second crop than of the first crop of alfalfa when the plants were young, but when the plants became older there was more present in first growth stems and leaves.

Soil type apparently was related to some changes which occurred in the moisture content of alfalfa as the growth period advanced.

Greater specific gravity was possessed by the expressed juice of alfalfa leaves than of stems.

The specific gravity of the juice of alfalfa stems increased for a period and then usually decreased to maturity. That of the juice of alfalfa leaves increased in an arithmetical progression during the growth period.

The specific gravity of the juice of both stems and leaves was noticeably greater in plants on heavy soil types.

The specific gravity of the juice of second growth alfalfa stems and leaves was much greater than that of the juice of first growth stems and leaves.

The specific gravity of the juice of the stems and leaves of both crops appeared to conform very closely to the calcium and magnesium content, showing that these cations were important in determining it.

The ratio of stems to leaves was about equal on the different soil types at the budding stage.

The calcium, magnesium, and moisture content of alfalfa stems and leaves varied at different hours during the day. This was also true of the calcium and magnesium contents and the specific gravity of the expressed juice of stems and leaves.

The percentages of calcium and magnesium in the tissue of stems and leaves varied almost inversely as the concentration of these in the juice of the stems and leaves at different hours of the day, probably because of differences in the amounts of juice present in the tissue as a result of changes in the moisture content.

The most satisfactory time to obtain alfalfa samples in order to eliminate

variations due to time of day lies between noon and 4 p.m. The least satisfactory time lies between 8 a.m. and noon.

REFERENCES

(1) Andre, M. G. 1907 Sur la composition des sucs vegeteaus extraits des tiges et des feuilles. *Compt. Rend. Acad. Sci.* [*Paris*]: 276–278.

(2) Austin, R. H. 1928 The effect of soil type and fertilizer treatment on the composition of the soybean plant. Thesis Graduate School Mich. State Col.

(3) Bryan, O. C. 1922 Effect of reaction on growth, nodule formation, and calcium content of alfalfa, alsike, and red clover. *Soil Sci.* 15: 23–29.

(4) Burd, J. S. 1919 Rate of adsorption of soil constituents at successive stages of plant growth. *Jour. Agr. Res.* 18: 51–72.

(5) Chibnall, A. C. 1924 A new method for the separate extraction of vacuole and protoplasmic material from leaf cells. *Jour. Biol. Chem.* 55: 333–342.

(6) Dinsmore, S. C. 1907 The composition of the alfalfa plant at different stages of growth. *Nev. Agr. Exp. Sta. Ann. Rpt.* 1907: 39.

(7) Gilbert, B. E., and Hardin, L. J. 1927 The current mineral nutrient content of the plant solution as a possible means of chemical control of optimum fertilization. *Jour. Agr. Res.* 35: 185–192.

(8) Gortner, R. A., and Harris, J. A. 1917 Notes on the technique of the determination of the depression of the freezing point of vegetable saps. *Plant World* 17: 49–53.

(9) Handy, J. O. 1900 The volumetric determination of magnesium. *Jour. Amer. Chem. Soc.*, 22: 31–39.

(10) Harvey, R. B. 1918 Hardening process in plants and development from frost injury. *Jour. Agr. Res.* 15: 83–111.

(11) Hoagland, D. R. 1920 Relation of concentration and reaction of nutrient medium to the growth and absorption of the plant. *Jour. Agr. Res.* 18: 73–117.

(12) Hornberger, R. 1882 Chemische Untersuchungen über des Wachsthum des Maispflanze. *Landw. Jahrb.* 11: 359–523.

(13) Kundson, L., and Ginsburg, S. 1920. Suggestions with respect to the measurement of osmotic pressure. *Amer. Jour. Bot.* 8: 164–170.

(14) McCool, M. M. 1926 The relation of the soil to the sap of plants. Mich Agr. Exp. Sta. Quart. Bul. 9, No. 2.

(15) McCool, M. M. 1925 The nitrogen content of alfalfa. Mich. Agr. Exp. Sta., Quart. Bul. 8 (3): 122-A.

(16) McCool, M. M., and Weldon, M. D. 1928 The effect of soil type and fertilization on the composition of the expressed sap of plants. *Jour. Amer. Soc. Agron.* 20: 778–792.

(17) McCool, M. M., and Millar, C. E. 1917 The water content of the soil solution as indicated by the freezing point lowerings of the roots and tops of plants. *Soil Sci.* 3: No. 2.

(18) Millar, C. E. 1926 Studies of the removal of nutrients from subsoil by alfalfa. *Soil Sci.* 23: No. 4.

(19) Morse, F. W. 1915 The effect on a crop of clover on liming the soil. Mass. Agr. Exp. Sta. Bul. 161.

(20) Russell, E. J. 1917 Soil Conditions and Plant Growth. London.

(21) Tottingham, W. E., Schulz, E. R., and Lepkovsky, S. 1924 The extraction of nitrogenous constituents from plant cells. *Jour. Amer. Chem. Soc.* 46:

(22) Whitney, A. L., and Richmond, T. E. 1926 The composition of biennial white sweet clover as related to soil enrichment. *Soil Sci.* 23:

(23) Widstoe, J. A. 1897 The chemical life history of alfalfa. Utah Agr. Exp. Sta. Bul. 48.

A NEW, SIMPLE, AND RAPID METHOD FOR DETERMINING THE MOISTURE EQUIVALENT OF SOILS, AND THE ROLE OF SOIL COLLOIDS ON THIS MOISTURE EQUIVALENT

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication December 3, 1928

In a communication (1) published nine years ago, the following statement was made:

> Water is one of the best indices of the physical characteristics of soil. Texture, structure colloidal and organic contents, surface activation, etc., tend to be revealed by the behavior of the soil toward water. Heat of wetting, moisture retentiveness, unfree water, etc., are mainly the resultant and expression of these characteristics.

After nine years of further study along these lines, the writer still believes that water affords probably the best index of the physical characteristics of the soil. The behavior of a soil toward water probably gives truer and more comprehensive composite information concerning the physical characteristics of that soil than the behavior of the same soil toward any other agent. This is probably due to two main factors: first, water, besides being the most natural and universal reagent, is also the chief natural agent by which the soil has been formed; second, most of the physical properties of the soil run parallel with its behavior toward water—for instance, the finer the texture of a soil, the greater is its hygroscopic water, absorption-adsorption of water, water holding capacity, capillary movement of water, evaporation of water, unfree water, wilting coefficient of plants, and shrinkage of soils. In the same parallel direction the greater the organic matter content is in a soil, the greater will be its hygroscopic water, absorption-adsorption of water, water holding capacity, capillary movement of water, evaporation of water, unfree water, wilting coefficient of plants, and shrinkage of soils. Here, therefore, the texture of mineral soils and the content of organic matter are not opposed in the behavior of a natural soil toward water.

On the other hand, if a single property of soils, such as plasticity, is used as an index of the general characterization of soils, we find that texture of mineral soils and organic matter content are opposed, and consequently plasticity and the other physical properties of soils do not run parallel, as in the case of water. For instance, the finer the texture is in a mineral soil, the greater will be its plasticity. On the other hand the greater the organic matter content is in a soil, the smaller will be its plasticity.

After the development of the hydrometer method (2, 4) for determining the colloidal content of soils and also for making a mechanical analysis of soils in only fifteen minutes, some single value factor was desired which would be simple, rapid, and reliable to accompany and complement the hydrometer method. Furthermore, it was desired to see whether the results of the hydrometer method could be summarized in a composite form in some single value factor.

In the belief, as already stated, that in the agent of water, or in the relationship of soil to water, resided the greatest possibility of discovering this single value factor, all efforts were concentrated in this direction.

The relationship between soil and water that was first considered, was the maximum total water holding capacity of soils. It was thought that if a method such as that of Hilgard (7), could give a reliable and truly comparable value of water holding power between different soils, it would serve as an excellent single value factor of summarizing most of the physical properties of soils. Unfortunately, however, Hilgard's method for determining the maximum moisture holding power of soils is most unreliable and extremely erroneous, and should not be used in its original form. The surface tension forces on the bottom of the cup tend to prevent a thorough drainage of the soil, and a considerable and variable amount of water is held back.

The next relationship of soil to water that was considered and carefully examined was the moisture equivalent factor as proposed and determined by Briggs and McLane (5, 6). This relationship would seem to afford a splendid single value factor. But unfortunately, here again, the method for obtaining the relationship is not accurate and probably does not give a truly comparable result for the soils of different textures. Here are the difficulties that have been encountered with the method at this laboratory: 1. In the case of heavy clays, especially the sticky type, the centrifugal force compacts the layer of soil to such an extent that the water is not thrown out but collects at the top of the layer. Consequently the results of such soils are not truly comparable with those of soils which allow the water to get out easily by the centrifugal force. That the results for different soils may not be truly comparable, would seem to be borne out by the examination of some data recently published by Professor Lebedeff (8). Professor Lebedeff employed widely different centrifugal speeds to obtain the moisture equivalent of soils. When soil 1 (table 3), which must be a clay, and soil 7, which is Sassafrass silt loam, were centrifuged for 50 minutes at 400 g (g is the acceleration of gravitation), soil 1 had a moisture content of 47.9 per cent and soil 7, 25.6 per cent. Now when these two soils were again centrifuged one minute at 70,000 g, the moisture content of soil 1 was reduced to 34.6 per cent, whereas the moisture content of soil 7 was reduced to only 7.1. By calculating the difference of moisture of the same soils at the two different speeds of centrifuging, it is found that soil 1 lost only 27.76 per cent more water when it was centrifuged at 70,000 g, while soil 7 lost 72.30 per cent. It is common knowledge that coarse textured soils

hold water with much smaller forces than fine textured soils. Consequently it would be logical to expect that the coarse textured soils would lose their water more easily at 400 g than the fine textured soils. Therefore, soil 7 should have lost proportionally much greater water at this force than soil 1 and at the centrifugal force of 70,000, soil 1 should have lost much more water than soil 7. But the results are just the opposite, and the explanation for this would seem to be that the clay soil (no. 1) becomes so compact as a result of the centrifugal force, that it is mechanically unable to give up all the water that it should under those forces, whereas soil 7 being of a coarser texture, does not present the same mechanical difficulty. Consequently it loses all the water that it should under those gravitational forces.

Several other single value factors were next considered, such as the wilting coefficient, hygroscopic coefficient, and vapor adsorption, but they were all finally abandoned as being impractical either because they were not sufficiently accurate and reliable, or because of the enormous length of time they required, or both.

It was finally decided to search for some new method to be used as a single value factor to accompany and complement the hydrometer method, a method that should still have water for its basis.

It seemed at the outset that a possible combination or modification of the maximum moisture content method and the moisture equivalent method might yield the desired new method. It was thought, therefore, that instead of applying centrifugal force to pull out the water from the soils, the same thing might be better accomplished by suction or vacuum forces. It was argued that the suction or vacuum forces would not compact the soils, and consequently the water would come out as easily in clay soils as in sandy soils, and the results, therefore, would be truly comparable for these different textured soils.

It is the object of this paper, therefore, to present a new, simple, and reliable method for determining the moisture equivalent of soils, which might be used as a single value factor and also to show what relationship the results of this method bear to the results obtained by the hydrometer method in the mechanical analysis of soils.

DESCRIPTION OF METHOD

The principle of the method is based upon the pulling out of water from soils by suction or vacuum forces. With this principle in mind one can use almost any method, provided it is standardized so as to give consistent and comparable results. The method that was worked out and finally adopted consists of connecting a Büchner funnel with a suction flask, placing a sample of soil into the funnel with a filter paper at the bottom, thoroughly soaking the soil with water, connecting the flask to the suction pipe on the faucet, and then allowing the suction force from the running water to operate on the soil for a definite length of time. The actual set-up of the apparatus is shown

in plate 1. The funnel is small—4.3 cm. in diameter and 2 cm. deep. The filter paper that fits this funnel is 4.25 cm. The funnel was about three-fourths filled with soil, then filled to the top with water. Water was added until the soil seemed to be completely soaked. Then the suction was applied to the soil and more water added to it until it was well settled. The suction pulled the water from the soil very rapidly, and after the first four or five minutes the water drained very slowly. For purposes of comparison, a period of 10 minutes, beginning when all the standing water had disappeared from the top of the soil, was allowed for all soils to drain under suction. Although the water did not stop draining at the end of 10 minutes, it slowed down so much that two minutes or longer were necessary for one drop to come through. Many trials showed that if a soil was drained for much longer periods, in some cases 50 minutes, the results would not be very different, provided evaporation was prevented.

It was found that it was not necessary to take a definite quantity of the various soils. For trials, the amount of soil used was varied considerably but the results did not show any definite relationship to the amount of soil used.

In order to prevent evaporation during the suction process, the funnel was covered with a moist thick cloth. This moist cloth served two purposes—prevented direct evaporation, and tended to moisten the air that was drawn into the soil.

The heavier types of soil have a tendency to crack when they are drained, and it would seem that this might affect the results. Investigation showed, however, that this cracking has no apparent effect on the results, as the same results are obtained when the cracks are prevented from occurring or being filled with extra soil.

Probably the two factors that are likely to cause the most error or disagreement in the results are the preparation of the soil sample and the temperature of the water.

As would be expected, it was found that very fine grinding of the soil sample increased its water retaining power, and in some soils much more than in others. The soil samples, therefore, should be used as much in their natural textural condition as possible. The practice that was followed and one that seems to be an excellent one, was to place lumps of the soil in the funnel and then let the water slack these lumps. In this way the soils tended to retain their natural texture, and errors arising from this factor were greatly diminished. Where it is necessary to screen, the soil should be broken up with the fingers.

Although it has been stated that the soils should be in as natural a condition as possible, it was found that they have to be dry to be used satisfactorily. This is due mainly to the fact that in the moist condition the soils do not slack (7) and consequently do not become thoroughly soaked. Hence, the soils should always be air-dried before using.

The temperature of the water, both in the soil and in the flask, has some

influence on the results. This would be naturally expected because temperature affects both the viscosity of water and the degree of vacuum produced. It is well known that the lowest exhaust pressure obtainable is usually considered to be limited by the vapor pressure of water. The best and most consistent results, therefore, were obtained when the temperature of the water remained about the same for the different experiments. Probably the best way to maintain an even temperature is to let the tap water run for

TABLE 1

Percentage of moisture equivalent, colloidal content, and relationship between these two sets of results for the various kinds of soil

SOILS	PERCENTAGE OF COLLOIDS	PERCENTAGE OF WATER	RATIO: $\frac{\text{WATER}}{\text{COLLOIDS}}$
	per cent	*per cent*	
Strongs sandy loam A_1	10.7	5.8	0.557
Strongs sandy loam B_1	27.00	14.02	0.519
Calumet sand	8.70	5.60	0.644
Michigan sand C	8.5	5.74	0.675
Michigan sand C	14.0	9.27	0.662
Fox loam B_2	18.08	11.95	0.657
Onaway loam A_1	11.69	7.42	0.634
Stalwart loam A_1	24.22	12.62	0.521
Ontonagon silt loam A_2	63.82	32.70	0.512
California Yolo clay loam	53.94	35.10	0.651
California Yolo clay	56.80	35.16	0.618
Brookston clay loam A_2	38.90	22.35	0.573
Greenville clay loam B	46.30	23.63	0.510
Cecil clay loam B	61.50	36.60	0.595
Manor schist loam B_1	46.00	31.75	0.689
Manor schist loam B_2	32.4	22.45	0.691
Michigan silt loam	36.00	19.00	0.528
Wabash clay	54.20	38.50	0.710
Cass clay	44.00	29.00	0.659
Minnesota Clyde silt loam	36.00	26.15	0.713
Bremer clay	55.40	38.20	0.689
Minnesota Carrington silt loam	51.00	35.00	0.686
Average			0.6224

a while until it reaches its normal temperature and then use this water for soaking the soil as well as for the suction purpose.

As already stated, the vacuum pressure attained in a water jet pump is considered to be limited by the vapor pressure of the water. The pressure or the force with which the water jet runs also affects the vacuum pressure. As a rule, however, the pressure of the water in any one water system is not likely to vary very considerably from day to day and if the running water is allowed to attain its normal temperature, there should not be much error

caused by this vacuum pressure system recommended here. In the different laboratories the pressure and temperature of the water may be different, but as long as they remain about the same for the different experiments, the comparison for the soils studied will be true.

The vacuum pressure that was obtained in the water system in this laboratory was about 20 mm. and remained quite constant from day to day. The temperature of the tap water was about 15°C. Consequently all the experiments received about the same degree of suction.

The method is quite rapid. The longest time in the procedure is taken up in soaking the soils. The length of time spent may be greatly reduced, however, by using two or three sets of apparatus, so that while one soil is being suctioned, one or two soils are being soaked. With the exception of some unusual soils, about 20 minutes is sufficient for them to soak quite completely (3).

Of course it is probably needless to state that this method is empirical, but an empirical method, if it is reliable reduces to a common basis and gives a true comparison, is as valuable as an absolute method as far as the real comparison is concerned.

It appears from the study this method has received, that it is quite reliable and gives a fairly true comparison of the moisture equivalents or the comparative water holding powers of the different soils. It also appears to give a truer comparison between the different textured soils than the Briggs and McLane method, and it is also simpler, more practical, and infinitely more available. If all the precautions stated above are observed, the results can be duplicated within about one per cent.

EXPERIMENTAL DATA

Table 1 shows the percentage of water that the different types of soils retain under the experimental conditions already set forth, the percentage of colloidal material of these soils as determined by the hydrometer method at the end of 15 minutes, and also the ratio of the percentages of moisture and of colloids for each soil.

The percentages of water and of colloids vary widely for the different soils, and both tend to be high with fine textured soils and low with coarse textured soils. The significance of these two sets of results, however, is revealed in the ratio figures. Here it is shown that when the percentage of water is divided by the percentage of colloids a ratio is obtained which, taking all factors into consideration, is remarkably close for all the different types of soils, such as coarse sand, heavy clay, high silt content, high organic matter content, no organic matter content, and high iron content. This ratio varies from 0.510 to 0.713, with an average of 0.6224. The majority of the soils have a ratio close to this average. If the vacuum pressure method for obtaining the moisture equivalent and the hydrometer method for obtaining the colloidal content would give perfectly true results for all soils, this ratio

would be even closer. It is known, however, that with some rather unusual soils these methods may not give exactly true results. For instance, if a soil is exceedingly difficult to disperse, the hydrometer method will not give its true colloidal content. Furthermore, an error of 2 or 3 per cent in either method makes quite an appreciable difference in this ratio. These variations in the ratio should not prevent the realization and acceptance of the fact that there is a real and unmistakably close relationship between the colloidal and the moisture contents of a soil.

The results also show that the controlling factor in the moisture equivalent is the colloidal content because there is no relationship between the coarse silt, sand and the moisture equivalent.

The moisture equivalent as obtained by the new vacuum pressure method and the colloidal content as obtained by the hydrometer method, besides showing a real and interdependent relationship, also show that the new moisture equivalent method is reliable, accurate, and tends to give true comparative results for the different kinds of soils.

This relationship reveals one more significant thing, that the hydrometer method for determining colloids, the heat of wetting method for determining colloids (2) and the vacuum pressure method for determining the moisture equivalent of soils are fundamentally sound and correct; at least they give true comparative results for all soils. When these three different methods, which are based upon entirely different principles, agree so remarkably well in their results on all kinds of soils, what better proof is needed of their fundamental soundness and correctness? The remarkable agreement of these three entirely different methods is exceedingly significant.

Since the relationship between colloidal content and moisture equivalent is so close, when one result is known the other can be calculated from the average ratio. For instance, one knows the colloidal content, the moisture equivalent can be obtained by multiplying the colloidal content with the ratio. If, on the other hand, only the moisture is known, then the colloidal content can be obtained by dividing the percentage of moisture by the ratio. Those who are using the hydrometer method can safely employ the factor 0.6224 for determining the comparative moisture equivalent of soils without experimentally determining this factor themselves.

From the foregoing experimental results, it becomes very apparent that both the new moisture equivalent method and the hydrometer method are sound and reliable for "single value" determinations. Of the two, however, the hydrometer method is by far the better. In the first place, besides giving the moisture equivalent of soils, it gives also the mechanical analysis of soils, which includes the percentage of sand, silt, and colloids or clay. When the mechanical analysis is known, much information is known about many other properties of soils. Secondly, the hydrometer method is more absolute than the new moisture equivalent method; that is, different workers will get the same result on any one soil under a variety of conditions if directions are

closely followed, whereas with the moisture equivalent method the different workers may not get the same result on any one soil, because they may be dealing with different temperatures and different vacuum pressures. The results of all soils in any single laboratory, however, will be truly comparable.

The hydrometer method for the study of soils offers greater opportunities and possibilities than have been realized.

SUMMARY

A new method has been developed for determining the moisture equivalent or comparative moisture holding power of soils.

The principle of this method is based upon the pulling of water from the soil by vacuum pressure forces instead of by centrifugal forces.

The method is simple, rapid, accurate, reliable, and infinitely more available than the centrifugal method.

The results obtained by this method show that there is a remarkably close relationship between the moisture equivalent and the colloidal content of soils as determined by the hydrometer method. There is, however, no relationship between coarse silt and sand and the moisture equivalent.

It is shown that the moisture equivalent or comparative moisture holding powers of the different soils can be indirectly determined by the hydrometer method.

It is also shown that the hydrometer method may be used to obtain "single value" factors for summarizing the various physical properties of soils.

The hydrometer method has inherently great possibilities for the study of soils.

REFERENCES

(1) Bouyoucos, G. J. 1922 Relation between heat of wetting, moisture equivalent, and unfree water. *Soil Sci.* 14: 431–434.

(2) Bouyoucos, G. J. 1927 The hydrometer as a new and rapid method for determining the colloidal content of soils. *Soil Sci.* 23: 319–331.

(3) Bouyoucos, G. J. 1927 Texture and structure of soils as influenced by chemical agents. *Amer. Soc. Agron.* 19: 788–797.

(4) Bouyoucos, G. J. 1928 Making mechanical analysis of soils in fifteen minutes. *Soil Sci.* 25: 473–480.

(5) Briggs, L. J., and McLane, J. W. 1907 The moisture equivalent of soils. U. S. Dept. Agr. Bur. Soils. Bul. 10.

(6) Briggs, L. J., and McLane, J. W. 1910 Moisture equivalent determinations and their application. *Proc. Amer. Soc. Agron.* 2: 138–147.

(7) Hilgard, E. W. 1914 Soils. New York.

(8) Lebedeff, A. F. 1928 Determination of the maximum molecular water capacity of the soil by means of centrifuging and characteristics of mechanical properties of soil by determining the maximum molecular water capacity. *Pedology* 1–2: 49–69.

PLATE 1

Main Apparatus Used in Determining the Moisture Equivalent of Soils

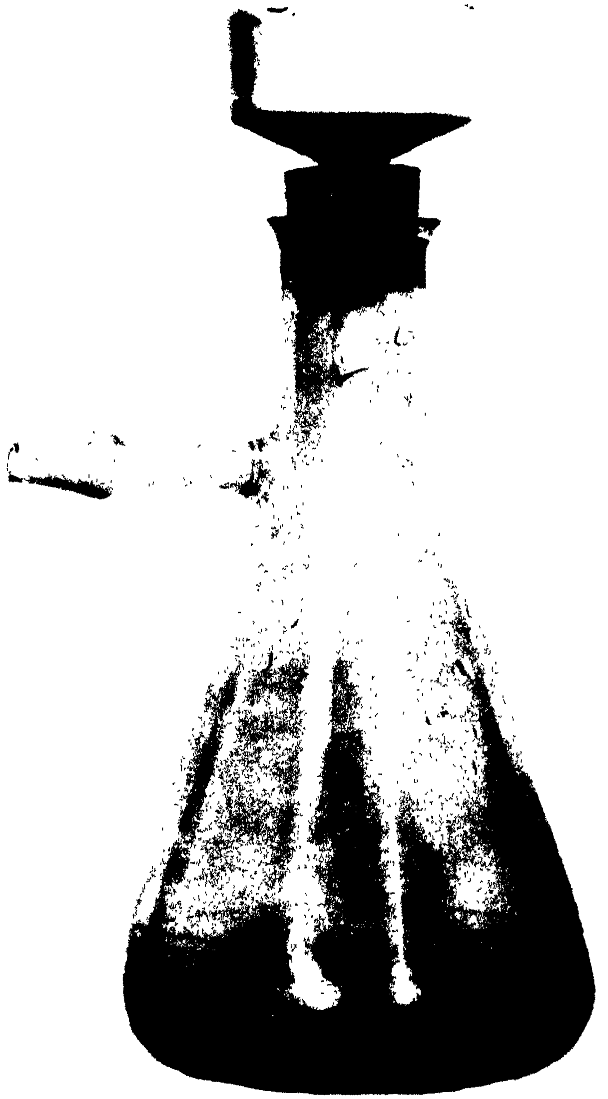


FIG. 1

REPAIR OF SOIL FILTER TUBES

G. J. LARSINOS AND A. B. BEAUMONT

Massachusetts Agricultural Experiment Station

Received for publication December 20, 1928

It is the common experience of soil analysts that the Chamberland-Pasteur unglazed porcelain filter tube used in making aqueous extracts of soils breaks rather easily. The break usually occurs near the juncture of the unglazed tube and the glazed nipple. The tube may be easily and inexpensively repaired by slipping a piece of stout rubber tubing of 20 mm. inside diameter

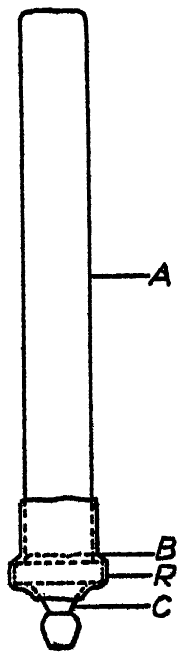


FIG. 1. SHOWING METHOD OF REPAIRING SOIL FILTER TUBES
A = unglazed filter tube; B = break; C = glazed nipple; R = rubber tubing

over the nipple and tube, as shown in figure 1. The air pressure used in filtering effectively seals the tube against leakage. Tubes so repaired are fully as serviceable as unbroken tubes and are less susceptible to further breakage. Chamberland-Pasteur filter tubes cost about \$2.65 each but can be repaired with about 5 cents worth of rubber tubing.

THE USE OF DEXTRINE IN THE ISOLATION AND IDENTIFICATION OF AZOTOBACTER CHROOCOCCUM

C. E. SKINNER[1]

University of Minnesota

Received for publication January 7, 1929

It is generally conceded that species of Azotobacter are fairly difficult to isolate in pure culture from the soil. I have in many cases found it exceedingly difficult to separate Azotobacter from certain non-spore-forming gram-negative rods (*Bacterium radiobacter?*), and from gram-positive spore-formers, using the ordinary Ashby's agar or other nitrogen-poor mannitol media. I have also found that many strains of Azotobacter showed either no blackening, or showed it only after prolonged incubation. Formerly I thought that I was justified in considering these non-chromogenic strains as species other than *A. chroococcum.*

Recently while using Baldwin and Fred's (1) nitrate dextrine medium in the study of many strains of the so-called *B. radiobacter*,[2] I noticed an intense jet black coloration within a week or so, often in a few days, when Azotobacter was planted on this medium. This fact led to the substitution of dextrine for mannitol in Ashby's agar, and it was found that after preliminary enrichment in Ashby's or Lipman's mannitol solution *Azotobacter chroococcum* could be isolated very readily from dextrine agar plates streaked from the enrichment flasks. Impure colonies were easily avoided. The dextrine agar slant cultures picked from the dextrine agar plates were again streaked on dextrine nitrate agar to test for purity. *B. radiobacter* and most spore-formers grow readily on this medium and any contaminant is easily noticed, since pure cultures of *Azotobacter chroococcum* showed only black colonies. Undoubtedly there are species of Azotobacter other than chromogenic ones, but as yet I have never isolated one which did not produce a black pigment on dextrine agar, particularly if nitrate was also present.

The use of dextrine nitrate agar thus not only facilitates the isolation of *Azotobacter chroococcum*, but also prevents one considering many strains as non-chromogenic. Many strains of *Azotobacter chroococcum* have been isolated in this way without the failures formerly encountered.

[1] Department of Bacteriology and Immunology.

[2] I inadvertently misquoted Baldwin and Fred in a former article (*Soil Sci.* 25: 195–205, 1928) as saying in effect that "this organism (*B. radiobacter*) can be readily distinguished from all strains of the nodule bacteria by the large amount of acid produced from dextrine by *B. radiobacter*." What they actually wrote was "*B. radiobacter* may be distinguished from any of the root nodule bacteria by its strong acid fermentation of dextrin," quite another thing, since only a possibility is implied, not a certainty as I attributed to them.

Recently I had a portion of Omelianski's (2) monograph on nitrogen fixing bacteria translated[3] and was surprised to find that Professor Omelianski and other Russian investigators, had been using dextrine for a number of years for the purposes enumerated, but I do not know of laboratories elsewhere where it is in use.

It was with the idea of calling the attention to this carbohydrate of any other workers who are not now using dextrine, that this note was written. Difco brand of dextrine was used, but no doubt other highly purified products would suffice.

REFERENCES

(1) Baldwin, I. L., and Fred, E. B. 1927 The fermentation characters of the root nodule bacteria of the Leguminosae. *Soil Sci.* 24: 217–230.

(2) Omelianski, B. L. 1923 Fixation of Atmospheric Nitrogen by Soil Microorganisms (Russian), Petrograd.

---

[3] I am grateful to Dr. Helen Sorokin for translating a portion of Omelianski's monograph from the Russian.

BOOK REVIEW

Laboratory Manual of General Microbiology with Special Reference to the Microorganisms of the Soil. By Edwin Broun Fred and Selman A. Waksman. McGraw-Hill Book Co., New York. 1928. Pp. 145, fig. 19.

In 1911 there appeared two excellent laboratory manuals dealing with soil bacteriology, those of Lipman and Brown and of Löhnis. Soon after, these were followed by three others, by Burgess, by Whiting, and by Fred. No field of bacteriology has been so well served with worthy laboratory manuals as has soil bacteriology, and of these manuals, that of Fred is in many ways the best. It is very widely used, and many of the younger workers got their first training from Fred's manual rather than from, or in spite of, text-books and professors. These books are now hopelessly out of date—that of Löhnis has been revised but not as yet translated into English—and it was with more than a little pleasure that teachers and investigators received the news that Fred's manual was being revised.

The new book, however, is more than a revision. In spite of the title, it is a soil microbiology manual, which for 1928 is a worthy successor to the 1916 book. It contains also many of the excellent methods summarized by Waksman and co-authors in Abderhalden's Handbuch. The emphasis in December 1928, is not primarily on "nitrogen fixation and all that sort of thing," but covers the whole soil population of microörganisms and their activities. The special interests of neither of the authors have received undue emphasis, but together they have made a splendid selection of culture media formulae and laboratory exercises. Like the 1916 book, this manual is divided into three parts, one of which gives formulae of 111 well selected media, another of which gives in outline the necessary qualitative and quantitative chemical methods, and the last part of which deals with laboratory exercises as such. In addition five pages of staining technique are given.

In spite of the excellent qualities of the manual, candor requires that certain small defects be mentioned which detract somewhat from its value. In general the book is exceedingly well arranged, but in a few places it is not. For instance, the preparation of phenoldisulfonic acid is given on page 60, but the use of it a few pages later. Likewise the aeration method of NH_3 determination is given on page 63 with other chemical methods, but the base exchange method on page 127 with the experiments. The preparation of methyl red is not described with the rest of the indicators. As was said, the selection of exercises and media is excellent. The reviewer, however, would like to have seen the more accurate Rothamsted methods and tables for counting soil protozoa given. Certainly the use of solid media for protozoa

should have been insisted upon. The new Foreman method, at once simple and accurate, for determination of amino acids, might have been given mention. Although, perhaps it ought not to be necessary, it might have been advisable to mention the possible loss of NH in each medium of a fairly high pH which contains an ammonium salt. There are very few misprints or mistakes. *Clostridium pastorianum* is the spelling originally used by Winogradsky. The term "thermostat" on page 24 is incorrectly used. It is doubtful whether the method of determination of hemicelluloses on page 77 is even approximately accurate. These points, however, are of minor importance and are perhaps partly due to the dual authorship.

Of course the practicability of a manual can be determined definitely only after it has been used for some time and has been tried out on classes of students. The reviewer ventures the opinion, however, that this one will prove, if rightly employed, as useful as Fred's 1916 manual did. No investigator or teacher who even dabbles in soil microbiology should try to get along without a copy of the manual by Fred and Waksman.

C. E. Skinner,
Department of Bacteriology and Immunology,
University of Minnesota, Minneapolis.

Volume XXVII April, 1929 Number 4

SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
New Brunswick, N. J.

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to Dr. Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

Soil Science is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Volumes begin with the January and July issues, respectively. The subscription price is $10.00 for Volumes XXV (Nos. 1–6 inclusive) and XXVI (Nos. 1–6 inclusive) January, 1928, to December, 1928, inclusive, United States, Mexico, Cuba. An additional charge per volume of 50 cents for subscriptions in other countries. *Back volumes* are supplied on orders for Vols. I to XIV inclusive, XIX to XXIV incl.—$100.00 per set.

New subscriptions are accepted to begin with the current issue, unless the subscriber gives instructions to begin the subscription with some other issue. If the subscriber desire, back issues of the current volume may be obtained when in stock. The publishers do not guarantee to supply back issues, however.

Renewal subscriptions. To avoid a break in your series, subscriptions should be renewed promptly on expiration. Otherwise it may prove impossible for the publishers to furnish the back issues missed, as only sufficient copies are printed of each issue to supply orders in hand.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; or G. Jervis Manton, 430 Lit. Collins St., Melbourne (and at Sydney and Brisbane).

For the British Empire, except Australia and Canada: Bailliere, Tindall & Cox; 8 Henrietta Street, Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann, Krausenstrasse 38, Berlin N. W., Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

Publishers of: *Medicine, Journal of Urology, Bulletin of the Johns Hopkins Hospital, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Physical Therapeutics, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thompson Research Laboratory, Journal of Biological Chemistry, Chemical Reviews, Journal of Dairy Science, Soil Science, Social Forces, Personnel Journal, Journal of Comparative Psychology, Comparative Psychology Monographs, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, American Speech, Childhood Education.*

STUDIES OF CERTAIN PHASES OF THE INTERRELATIONSHIP BETWEEN SOIL AND PLANT: I. AVAILABILITY OF MINERAL PLANT NUTRIENTS IN RELATION TO THE DEGREE OF DISPERSION[1]

WALTER THOMAS

Pennsylvania State College

Received for publication December 29, 1928

A. INTRODUCTORY

The subject matter of this paper deals with a field of research that, although of the greatest importance, is still in a state of flux. Notwithstanding the existence of a voluminous literature on the permeability to and absorption of substances by living animal and plant cells, there is little exact knowledge. And to the complex of factors now to be considered that of colloidal behavior must be added.

Colloidal solutions have been *arbitrarily* defined on the basis of the size of the dispersed particles ($1\mu\mu$ to 100–$200\mu\mu$). There is, however, no infallible test for colloidality. Even the ultra microscope and cataphoresis tests may fail under some conditions and with some colloids (82). Dialysis experiments, moreover, may not always be specific, for artificial semi-permeable (collodion) membranes have been prepared that are permeable to one crystalloid (e.g., NaCl), but not to another (e.g., $NaNO_3$) (7, 8); to starch and dextrin, but not to night blue and other colloidal dyes; to colloids but not to crystalloids (45, 46).

1. *Theories of permeability*

It is not definitely known what makes a membrane semi-permeable. According to Kahlenberg (45) an absolutely semi-permeable membrane exists only in theory. From their comprehensive studies on the conditions governing the permeability of certain physiological membranes for various *electrolytes*, Michaelis and Weech (59) conclude that it is fallacious to speak of a *definite permeability* of any type of membrane for electrolytes, since in their experiments the behavior towards the passage of electrolytes was found to depend on a variety of conditions. Thus, these investigators showed that by changing the character and magnitude of the force driving the ions across the membranes

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as scientific paper No. 462.

and, according to the history of previous treatment of the membrane, the whole character of the so-called specific permeability for ions of the membrane could be varied *without any substantial change in the structure, chemical composition or pore size of the membrane.* And Höber (39) is of the opinion that membranes possess both a "physiological" as well as a "physical" permeability, because the mechanism of penetration into membranes by the different classes of substances, such as organic substances, salts, acids, bases and water, appear to be so distinctly different with each group. The older definitions, therefore, of semi-permeable membranes break down entirely.

All theories of the permeability of *living* cells assume the existence of some sort of a "membrane," although differences of opinion exist as to its nature. The more prominent of the older conceptions of the factors determining the penetration of substances into living cells are Overton's "lipoid" theory (68) and Clowes' conception of the cell membrane as an "emulsion" (13); but it has been urged (41) that no one theory is capable of explaining all the peculiarities of cell permeability such as, for example, the penetration of water-soluble substances like sugar and which are not lipoid-soluble. Moreover, most of the theories break down in accounting for functional changes in permeability, that is, the permeability of the plant cell is not constant. Nevertheless, most authorities agree that there can be little doubt that "lipoid" solubility is a property favoring ready entrance into living cells, the sole exception to the rule known at present being the cases presented by a *colloidal dye* in the presence of plant cells (39).

2. *What constitutes true colloidal behavior?*

One of the main difficulties to be faced in an evaluation of the factors determining whether or not colloids can be absorbed by plants is in characterizing what constitutes true colloidal behavior. Although colloids have been arbitrarily defined on the basis of the size of the dispersed particles, there is no agreement on what constitutes true colloidal behavior. Loeb (55) premises that ampholytes like the proteins show colloidal behavior only when the protein ions are prevented from diffusing through those membranes or gels that are readily permeable to the ions of ordinary salts and that the distinction between crystalloids and colloids, as Graham (29) conceived the difference, is not tenable. If all ampholytes behave like proteins (as doubtless they do), then, according to Loeb's view, they are true colloids only at the isoelectric point, i.e., when they exist as non-iogenic substances. The ions of Loeb, then, that show true colloidal behavior are the same as the micellae of McBain that originate through the electrolytic dissociation of neutral colloidal systems.

If, as it would appear, colloidal behavior is a surface phenomenon, the clearest conception of the behavior of colloids is obtained, as Wilson suggests (96), by regarding the particles as having more of their valences directed from the molecule and, therefore, unsatisfied (i.e., residual valency). The consequence of the existence of such free valences will be to cause substances

approaching their surfaces to combine and give up their charge to the colloid. The colloid may also acquire its charge by ionizing itself (22).

As Oakley (65) well expresses it, the colloidal condition may well be conceived as a compromise due to the differences in the affinity of the un-ionized and ionized groups for water, the boundary of the ionized ions being conditioned by the balance existing between the osmotic and electrometric forces. The membrane defined by the limits of this boundary will be governed by the Gibbs-Donnan equilibrium, the non-diffusible ions being the anions or cations that are partners to the colloidal cations or anions, resulting in a potential difference (P.D.) between the surface layers and the bulk of solution. This P.D. is found by equating the electrical work done by moving one equivalent of ions across the membrane to the work done against osmotic pressure, i.e., to $\frac{R.\,T.}{F}$ log y/x, where y/x are the concentrations of the negative ions on the two sides of the membrane.

3. On dialysis experiments

But all of these, conceptions, of colloidal behavior fail to explain much that is pertinent to our problem. For example, does dialysis increase or decrease the stability of sols? Hardy (35) maintains that dialysis as a means of isolating unstable or insoluble hydrous colloidal matter is open to criticism on the grounds that it progressively removes adsorbed stabilizing (peptizing) ions, thus destroying equilibrium conditions. The experiments of Thomas and Frieden (83) furnish an excellent example of this. There is a wide gap in our knowledge as to the causes operative in producing stability of sols and, therefore, of their exact composition. Take ferric hydrosol as an example. Thomas and Frieden (83) claim that the ferric oxide sol is represented by the formula xFe_2O_3, $yFeCl_3$, and that it exists in solution as the complex ions $xF_2O_3 \cdot Fe^+$ and Cl^-. But additional corroborative evidence is lacking to support this view. Indeed, Sorum's investigations (78) established beyond much doubt that stable hydrosols giving no test for Cl^- can exist. From Beans and Eastlack's work (3) it might be deduced that the ferric oxide sol in Sorum's experiments is stablized by the small amount of Fe in solution (for the oxide is soluble to the extent of 1 p.p.m.), or, possibly, it might be stabilized by the hydrogen ion from the ionization of the water on its surface.

From the foregoing it will be realized how fragmentary is our knowledge of the facts of permeability and how impossible it is in the present state of our knowledge to formulate any immutable laws of membrane action. This condition, however, may not prevent us from critically examining in detail the available experimental evidence bearing on our problem, to weigh the facts and to interpret them according to the scientific method. This we shall proceed to do.

B. EXPERIMENTS WITH ARTIFICIAL MEMBRANES, ISOLATED CELLS OR TISSUES AND ABNORMAL PLANTS

1. *Artificial membranes*

a. Gurchot's theory of colloidal penetration. The suggestive researches of Gurchot (31), indicating the mechanism of the reversible permeability of copper ferrocyanide membranes, have given additional information on the possible mechanism of penetrability. His copper ferrocyanide membranes give all evidences of behaving as a dynamic system. He concludes that the whole question of the action of his membranes involves adsorption, coagulation and peptization. That some form of adsorption, or, perhaps, solution of the septum takes place is now widely accepted. This is the basis of Overton's theory (67) and is very strikingly demonstrated in Kahlenberg's experiments (45, 46) with rubber damask and lanoline silk membranes and in Overton's own experiments (67) on animal and plant cells.

It is possible that a satisfactory working hypothesis of the behavior of *artificial* membranes may be worked out in the future on the basis of reduction or neutralization of the charge upon their colloidal surfaces. Penetration of the membrane by colloids would, if such a theory is substantiated, be due to membrane coagulation due to selective adsorption as a result of differences in potential set up at the interface between the solid and liquid phases in accordance with the Gibbs-Donnan law. It would serve to explain why substances pass through a membrane at certain times that do not do so normally. Gurchot's theory of membrane action does not imply that penetration is always accomplished by means of selective adsorption and coagulation. On the contrary, as he points out, under some conditions a membrane may act like a sieve for salts in dilute solution, because in this case they will, if the solutions are dilute enough, go through the membrane without coagulating it. This is entrance by osmosis. But when the concentration is raised the charge of the membrane will, if the cation is absorbed more than the anion, be neutralized and coagulation will, therefore, follow, in which case the salts or ions pass through by diffusion as through a dialyzing membrane. Indeed, Weech and Michaelis (92) are disposed to account for the difference in the diffusion rates of the non-electrolytes examined by them to the varying sizes of the pore channels of the membranes.

From Gurchot's results it can scarcely be questioned that artificial membranes, like the potassium ferrocyanide septums used in his experiments, may, under certain conditions, permit the diffusion of colloids; but the experimental demonstration and proof of colloid penetration in the case of normal living cells, as will be shown, is less convincing.

b. Equilibrium of colloidal and molecularly dispersed phases. There is, moreover, another possible mechanism that must not be overlooked. If, as it is reasonable to suppose from the hydration theory (96), that a fractional portion of all sols are in true molecular solution, penetration of colloids into

cells would take place, preferably by the entrance of that portion of the substance which is in true solution. The disturbance of the equilibrium produced by the diffusion of solute would enable more of the colloid to go into true solution. Evidence for this view is supported by the work of Foerdi and also of Fredenthal as recorded by Bechhold (4). From the observation that after ultrafiltering pure starch "solutions" a given fraction of the "solution" always passed through his membranes, the former concluded that for every concentration of the starch solution a balance existed between the larger particles and the molecularly dissolved starches. This view is upheld by Fredenthal who found that soluble starch produced a definite lowering of the freezing point (F.P.). We shall have occasion to discuss this conception later. But the experiments cited cannot be regarded as final, for marked differences existing in the membranes used by different investigators might vitiate comparisons unless the results are expressed in terms of "diffusion coefficients" (92). There is little doubt that differences in structure and composition of membranes, together with factors already stated (59) have contributed not a little to the difficulties presented in interpreting much of the experimental work on the ions present in the soil solution and which have been based upon dialysis experiments with collodion membranes. The confusing results on aluminum as a factor in soil acidity may be cited as an example (21, 43, 57).

2. *Living isolated cells and abnormal plants*

a. The absorption of fats. The work of Czapek (20) and Schmidt (77) on the penetration of fine emulsified fats is frequently cited in support of the theory of *colloidal absorption* by living cells. A critical investigation to test this theory was recently undertaken by Rhine (74). Schmidt's technique was repeated. When filter paper saturated with various oils colored with Soudan III were inserted into longitudinal incisions in starved pea seedlings, Rhine found that both *free fatty acids and neutral oils* entered the cytoplasm from the intercellular spaces and appeared in the cytoplasm in the form of small droplets. From these experiments he concluded that the penetration was a physical matter depending in part upon the viscosity of the oil. When, however, the tests were made with *emulsions of linseed fatty acid in water*, no penetration into the cytoplasm took place. Further investigations to explain this discrepancy disclosed the fact that the penetration of the fats and oils into the cells was due to a water deficit in the walls of the starved etiolated pea seedlings, for when tests were conducted with normal seedlings well supplied with moisture in air and soil no penetration took place.

Moreover, comparing the respiratory quotients in the hypocotyls of fatty and starchy seeds for the purpose of determining the type of food used by the germinating pea seedling, Rhine rightly concluded that the form could not be fat, since the fatty seed hypocotyls were being furnished carbon in the same state of reduction as were those of the starchy seeds. Other experiments

showed that no diffusion concentration gradient of fats existed, nor were fats found "en route" in the cell walls in tissues through which formerly they were supposed to pass.

These critical investigations of Rhine appear to offer evidence that fat emulsions do not enter normal plant cells, but rigid experimental proof using animal cells has not been forthcoming.

Bechhold (4) is authority for the statement that fats are found in the truly soluble form (e.g., soaps) only at the time when they pass through the wall of the intestine, after which they immediately regain their colloidal condition. Unfortunately, he cites no experimental evidence. If his view is substantiated, the mechanism of penetration in such cases might be comparable with Witzemann's conception (97) of the catalytic function of the leucoplasts in the condensation hydrolysis equilibrium involved in the formation and mobilization of starch and glycogen, the equilibrium being controlled by the second law of thermo-dynamics.

b. The penetration of dyes. Such an authority as Jacobs (41) states that many of the investigations on the penetration of acid and basic dyes (colloidal as well as non-colloidal) are worthless, partly on account of faulty technique and partly due to too broad generalizations from results on specific types of cells. Of the investigations relative to the conditions determining the penetrability of dyes, Ruhland's work (76) acquired prominence because his conclusions challenged Overton's view that "lipoid solubility" was the greatest factor determining ease of entrance into living cells. Ruhland demonstrated beyond doubt that not only the basic or acid nature of the dye but also, in the case of plant cells at least, the size of the molecule was the important factor. Colloidal dyes did not penetrate whereas non-colloidal dyes passed through. Höber (38) and more recently Gaedertz and Wittgenstein (23) have also confirmed Ruhland's conclusions (76).

However, it would be dangerous to infer that, because certain dyes having large molecules do not penetrate living cells, this is the determining factor in penetration. Beyond a certain limit this does not seem to hold true. This is exemplified by the results of permeability experiments with certain organic substances in which it has been shown that the size of the molecule may be of less importance than the presence of non-polar groups (51), for it is known that the introduction of such non-polar groups as $-CH_3$, $-C_2H_5$, and $-C_6H_5$ in place of hydrogen into the fatty acids increases their permeability (41).

C. CULTURE AND FIELD EXPERIMENTS WITH PLANTS

1. *Distinction between permeability and absorption*

There is, in the literature, some confusion of the terms permeability and absorption. As Osterhout (66) has shown, the terms are not synonymous, because if ions are removed by reactions occurring in the cell or in the intercellular spaces, considerable absorption of the nutrient may take place,

although the true permeability of the substance might be quite low. Accordingly, with the same permeability, the amount of substances absorbed may vary greatly, depending on conditions. In the following discussion on the absorption of nutrients relative to the degree of dispersion this distinction should be borne in mind.

2. *The arguments advanced against the solution theory of plant nutrition*

Comber's stimulating paper (14) on the possible rôle of colloids in plant nutrition has attracted considerable attention. This authority rightly objects to "the tendency among many soil chemists to take so many things for granted without inquiring scientifically and experimentally into their truths" (17). He feels convinced that the assumption that material must be in solution in the soil before it can be of any use to the plant is quite unproved. In modification of present views, Comber postulates that the colloids of the soil play an important rôle by being directly absorbed by plants through the formation of a one phase system between the colloidal coating of the plant root hairs and the colloidal coating of the soil particles. Hence, according to this hypothesis, part of the nutrients absorbed on the soil surfaces are mechanically incorporated by the root hairs. Nutrients may in this way be absorbed without the necessity of going into solution. If this view is true it would follow that diffusion of salts in the soil, especially those of phosphorus and of iron, would not be an important factor in plant nutrition. It should be added that an alternative hypothesis to that of the direct absorption of colloids is also put forward by Comber (14), viz., that the organic excretions of the roots may exert a dissolution effect on the particles.

Clearly, this conception has an important bearing on soil solution studies and soil analyses, on which account rigid proof should be forthcoming before it is accepted. The arguments advanced in favor of the proposed modification will now be critically examined.

a. The relation of the composition of the soil solution to the mineral elements taken up by plants. It has been maintained on the basis of observations of Hall (33) and of Ramann (73) that, since the ratio of certain mineral elements assimilated by plants to the water transpired is greater than the ratio of these same elements to the water in which they are dissolved in the soil solution, therefore, the crop does not derive all its mineral matter by the simple flow of the soil water by osmosis into the roots. Accordingly, the examination of the soil solution to ascertain what soluble substances are present is no criterion of what a particular plant can get out of the soil.

This conclusion cannot be valid, for, as will be shown, the premise is unsound: (*a*) Based on a calculation of the amount of K_2O taken up by a clover crop to the water transpired, Hall claims that the concentration of K_2O thus obtained, viz., 0.006 per cent, is greater than in solutions obtained from the soil. That such a limit may not hold generally is shown by many analyses of the writer (84) of the soil solution obtained by displacement methods (52, 69)

from a large number of orchard soils in Pennsylvania in which the K_2O content was found to be 0.0078 to 0.0084 per cent. (*b*) Additional evidence is furnished from the carefully controlled and exhaustive experiments of Muenscher (61) and also of Prat (72) which provide rigid proof of the fact that transpiration does not play an important rôle in the supply of nutrients to the plants. They have shown conclusively that the quantity of an element absorbed by a plant bears no relation to the water absorbed and lost by transpiration. In other words, Muenscher and Prat have proved that plants can absorb water and solutes differentially, the quantity of an element absorbed being dependent upon growth as influenced by humidity and light intensity, rate of transpiration and concentration of solution. Hoagland (37), moreover, has shown that the rate of absorption is also dependent on the nature of the ions.

These conclusions have also been experimentally substantiated by Curtis' investigations (18). Transpiration, therefore, cannot have much direct influence on the movement of solutes. Chemical change in the cells is of far greater influence than the mere mechanical effects of transpiration (19).

b. The absorption of silica by plants. The experimentally established fact of the absorption of SiO_2 from silica gels has also been produced as evidence in favor of the theory of colloidal penetration. The researches of Pfeffer (71), Gregoire (30), and especially of Jennings (42) leaves no room for doubt that silica when supplied either in the gel or sol condition is absorbed. Jennings used freshly prepared silica gels from sodium silicate, which were added to nutrient solutions in which wheat plants were grown. Analyses of these plants with the controls showed definite and relatively large absorption of silica.

But the conclusion that the absorption necessarily takes place in the colloidal condition *per se* is not valid. The facts, as we shall see, are also consistent with the solution theory.

(*1*) *The "solubility" of silica.* Truog (87), skeptical of the claim of the direct absorption of colloidal silica, offers as evidence against it that silica is soluble to the extent of 430 p.p.m. But difficulties at once arise with respect to the connotation "solubility." Lenher and Merrill (50) give 428 p.p.m. and Gile and Smith (27) give about 92 p.p.m. as the solubility of silica sols (silicic acid) in conductivity water. The former used S & S filter paper No. 589 and the latter Chamberlain filters. The researches of Joseph and Hancock (44) indicate a solubility of 32 p.p.m for anhydrous silica.

All these investigators are careful to qualify their use of the term "solubility" by the explanation that all or part may be present as a finely dispersed sol rather than a true solution. It is, therefore, all the more interesting to note that more than twenty years ago Mylius and Grosehuff (63) by treating sodium silicate with hydrochloric acid prepared an unstable metasilicic acid that passed through a semi-permeable membrane.

Norton and Roth (64) also conclude that silicic acid may exist in true solution. The presence of definite silicic acids, such as orthosilicic acid(H_4SiO_4),

in silica hydrogels and hydrosols, having the composition $xSiO_2 \cdot yH_4SiO_4 \cdot zH_2O$, in which the quantities of water and silica might vary from a true solution of silica in water through the highly dispersed gels, was indicated.

The writer (84) found that the silica in soil solutions obtained by displacement methods (69) dialyzed through collodion membranes prepared according to the method of Brown (7). McGeorge (57), too, found dialyzable forms of silica in all the Hawaiian soils examined and "little or no colloid forms." In the writer's experiments the dialysates contained 30 to 43 p.p.m. SiO_2. Moreover, owing to the well-known effect of the coagulating effect of ions, it is difficult to understand how, in the usual procedure for the analysis of clays, the sol could possibly remain stable in the presence of such large quantities of electrolytes, if the silica is not molecularly dispersed.

It is possible to interpret these results in accordance with the view presented by Hardy (34), viz., that silica may become soluble by the splitting off of small units of the adsorption complex, $xSiO_2 \cdot yH_2O$, owing to the ability of the "combined" water molecules to ionize.

The existence of pure silica hydrosols and hydrogels, as already mentioned, has been questioned. Hardy (34) concludes that the weight of evidence favors the view that hydrous silica is an ampholyte possessing an isoelectric point well on the acid side (pH 6.4), and records Williams' conclusions (95) that silica gel seems to possess a reticulate structure which when saturated contains water in two phases, the one absorbed at the surface of the material comprising the gel framework and the other filling the intermicellar spaces.

From the facts presented above, it would be unscientific to infer that because an increased absorption of silica by plants is found when silica gels are added to nutrient solutions absorption of the colloid takes place *per se*. The facts are obviously equally consistent with the simpler view of absorption from true solution.

(2) *The effect of silica gels on the absorption of other ions by plants.* Finally, it is of interest to inquire into the causes producing an increased absorption of phosphorus from rock phosphate in the presence of silica gels. Lemmermann and Weismann (49) attributed the increased yields to an increased assimilation of silica. This view was criticized by Gile and Smith (27), who contended that the beneficial action of silica gel on the growth of plants is to be attributed to the effect of silica on the solubility of rock phosphate. There is yet another possible explanation, viz., that derived from the application of the Gibbs-Donnan equilibrium law to the distribution of ions on the two sides of the semi-permeable membrane. This latter view is supported by the work of Butkewitsch and Butkewitsch (9), who have supplied experimental evidence on the influence of the presence of a non-diffusible ion (silica) upon the absorption of diffusible ions by plants. The application of the Gibbs-Donnan membrane equilibrium theory will be discussed in a subsequent paper.

c. The absorption of iron by plants. Comber (14) is unable to reconcile the absorption of iron by plants on calcareous soils with the solution theory.

This view is based on the insolubility of ferric oxide. It should be noted, however, that iron may exist in soils in comparatively large amounts in the *lower state of oxidation*. Thus, Vanstone (89) records the existence of iron in soil from the Folkstone Beds of England as the soluble and available ferrous phosphate.

Owing to the weakly basic character of Fe_2O_3, the ferric carbonate formed in calcareous soils is unstable, undergoing hydrolysis with formation of the sol $xFe_2O_3 \cdot yH_2O$, or under some conditions, a precipitate of ferric hydrate (12). Hydrous ferric oxide is an amphoteric electrolyte with a pH probably of about 6.5. Its basic properties, however, outweigh its acidic properties and the "ferrites" so formed are quite unstable. Its acidic dissociation, $Fe(OH)_3 \rightarrow 3(OH)^- + Fe^{\cdot\cdot\cdot}$, therefore, would, as recorded by Hardy (34), be accompanied by the formation of the irreversible mono-hydrate. The precipitated hydroxide has a low solubility in water (1 p.p.m.); if, however, conditions permitted the rate of absorption even at this low concentration to be high, enough Fe might still be furnished for normal metabolism by the disturbance of equilibrium, as already discussed (p. 253). The investigations of Gile and Carrero (25), who showed that soils which yield iron sufficient for the growth of plants may not show a detectable amount of iron in the water extract, may be cited in support of this view. The experimental evidence, without exception, in culture solutions has shown that only traces of iron need be present in the solution for normal growth.

If, on the other hand, Joffe and McLean's conclusions (43) from laboratory experiments are applicable to soil conditions, then we must infer that iron cannot occur in molecularly dispersed solutions in normal soils and very little in the sol state, since no $Fe(OH)_3$ in molecularly dispersed solution was found by them higher than pH 3 to 4; and that even in the presence of SO_4 ions no $Fe(OH)_3$ sol existed above pH 3.8. Apparently, however, in the presence of NO_3 and Cl ions, the $Fe(OH)_3$ sol could exist as high as pH 5.4. These results indicate the importance of the consideration of the influence of the other ions in the soil.

Since the criterion of the sol condition used by Joffe and McLean was non-diffusibility through collodion sacs, this criterion, as was indicated in the earlier part of this paper, may invalidate the sharp line of distinction made by these authors between true solution and the sol and gel conditions.

It would be of interest to correlate Joffe and McLean's results with Gile and Carrero's culture experiments (26), in which these authors show that colloidal iron that was not permeable to a parchment membrane was available to plants in an acid medium and to a limited extent in a neutral medium but not when $CaCO_3$ was added. Unfortunately, Gile and Carrero do not give the pH of their solutions.

In an earlier paper (25) Gile and Carrero, comparing the availability of dialyzed iron with ferric chloride to rice plants in culture solutions, conclude that rice could not assimilate "colloidal iron" but that the plants absorbed the

fraction of the iron that was molecularly dispersed from the dialyzed ferric oxide sol.

Hardy (35) believes that complex coördinated metal anions may readily penetrate and suffer translocation in the plant. This belief is founded on the results obtained by many investigators who have used different iron compounds in nutrient culture experiments; but, as there is no definite information on the composition of these complex organo-metallic compounds (36), the assumption that they are absorbed by plants seems unwarranted. There can be little doubt that the complex ferro-citrates and ferro-tartrates, as obtained by extracting iron oxides with citric and tartaric acids, are colloidal and that on standing they gradually undergo hydrolysis and precipitate out; but there is still some ferric ion in solution even after 60 days (36). In this connection it is interesting to note that analysis by the writer of a sample of Merck's "dialyzed iron containing 5 per cent Fe_2O_3" gave, after coagulating with $MgCl_2$ and filtering, 0.5 per cent of chlorine. Tested with potassium sulphocyanate the filtrate gave a slight blood red color. The presence, therefore, of some Fe in solution is indicated. It follows that the colloid-solution equilibrium hypothesis advanced earlier in this paper (p. 253) may also be applied to explain the penetration of iron (and aluminum) into cells. According to this view we would have:

$$\text{Colloidal } Fe_2O_3 \cdot xH_2O \rightleftarrows \text{dissolved } Fe(OH)_3 \rightleftarrows Fe^{\cdots} + 3(OH)^-$$

Relative to the absorption of other ferric sols by plants the experiments of Parker (70) show that colloidal ferric phosphate hydrosol, that was non-diffusible through collodion sacs, was not available to plants.

Field experiments on the subject show strange contradictions. Thus, Gile and Carrero's field experiments on calcareous soils indicate that iron-induced chlorosis may result in plants growing upon them. We are, therefore, on the horns of a dilemma. Iron is available to plants on the chalk soils of England, but in the calcareous soils of Hawaii it is not available in sufficient amounts to prevent chlorosis. The results on the Hawaiian soils do not support the explanation offered (14, 35) for the absorption of iron, viz., the formation of *organo-compounds* with such substances as sugars, starches, certain proteins, etc.

The conclusion seems inevitable, therefore, that more facts are needed before any claim to the absorption of colloidally dispersed iron by plants can be made. All the facts can readily be explained on the basis of the iron of soils being absorbed in the molecularly dispersed state.

d. The availability of mineral phosphates. If the phosphorus applied both in the "raw" mineral form and as superphosphate is present in the colloidally dispersed phase, it would not be possible, as many investigators have done, to find a correlation between the amount of water-soluble phosphorus, the amount of phosphate applied and the response of crops. In what follows, we shall discuss some of the salient points with reference to the conditions rendering the phosphorus of mineral phosphates available.

(*1*) *Superphosphates.* In a very ingenious discussion of the availability of phosphorus, Murray (62) points out that the compounds into which the soluble phosphates are converted by reversion in the soil will consist of particles of different sizes; some, therefore, will have larger and some smaller *surfaces.* As the particles below the critical stage (circ. $200\mu\mu$) representing the transition to the colloidal state will undergo comparatively rapid solution, it follows that the rate of absorption of ions by the plant will be determined by the number of particles below the critical stage.

Murray cites the law of diminishing returns, which Mitscherlich and Baule (60) have attemped to express as a fundamental law, in support of this view. Thus, when the particles below the critical stage are formed in such large numbers that equilibrium exists between the rate of absorption by the plant and the rate of solution, no appreciable difference in the yield would be expected to occur by the addition of more fertilizers. This is what happens in most cases, although exceptions to the general rule are known (48).

Murray's views on the formation of solid particles are based on the hydrolysis hypothesis. The investigations of Cameron and Bell (10) lead to the conclusion that the addition of mono-calcium phosphate to the soil results in the formation of the secondary salt $CaHPO_4 \cdot 2H_2O$.

$$CaH_4(PO_4)_2 \cdot H_2O + H_2O = CaHPO_4 \cdot 2H_2O + H_3PO_4.$$

They concluded that no definite compound existed intermediate between $CaHPO_4 \cdot 2H_2O$ and $Ca(OH)_2$, but only a series of solid solutions. Austin's (1) results on the electrometric titration of $Ca(OH)_2$ and of $CaCO_3$ with mono-calcium phosphate are essentially in accord with the views of Cameron and Bell. Both Warington's (91) and Bassett's (2) investigations, however, indicate that only the tricalcic phosphate $Ca_3(PO_4)_2$ and the hydroxy-apatite $Ca_3(PO_4)_2 \cdot Ca(OH)_2$ can be in stable equilibrium with an aqueous solution, and that the latter is the only calcium phosphate that can permanently exist under normal soil conditions. Which of the foregoing conclusions apply to the actual conditions in the soil, it is impossible to say. All that is certain is that calcium mono-phosphate is converted into a substance (or substances) that is very much less soluble than the original substance and that, possibly, the conversion may be into very insoluble forms. Murray's deductions are, therefore, in no way irrational. But the actual conditions existing in the soil may not be capable of explanation wholly by solution forces, as he supposes; for, apart from the possibility of the formation of aluminum and iron phosphates in acid soils, there are unquestionably other forces operative, in addition to those pointed out on page 263, to disturb the equilibrium conditions, such as those due to absorption of the PO_4 ions by the soil colloidal complexes. The tenacity with which these absorbed *ions* will be held will vary with the potential differences developed and, therefore, upon other cations and anions present in the absorption complex (16, 79, 81). The concentration of the PO_4 on the colloidal surfaces will be determined by the Gibbs-Donnan equilibrium law.

The experimental work in support of these facts is considerable, of which some only will be cited: (*a*) The PO_4 ions of superphosphate may be so strongly absorbed by some soils that acids may not dissolve more phosphorus from such soils treated with superphosphates than from soils supplied with rock phosphate (98). (*b*) In field experiments it frequently happens that as much as 20 per cent of the applied phosphorus is not recovered by the crop (27). Wheeler and Adams (93) concluded from their experiments on peat and muck soils that "saturation" of the soil colloids was necessary before additions of phosphates became effective.

Gordon and Starkey (28, 80), working with artificial iron and aluminum gels, have shown, as would be expected from theoretical considerations, that the greater the hydrogen-ion concentration of the surrounding solution, the lower the absorption of PO_4 ions and vice versa. Nevertheless, leaching of phosphorus may occur. A slight loss of phosphorus from a loam soil derived from Triassic Red Shale has been noted in the New Jersey Station cylinder experiments (5), especially in the larger phosphatic applications. On some North Wales shaly loams and even in some heavy clay loams Robinson and Jones (75) found considerable leaching of phosphorus—applied as slag—into levels below 18 inches, in a region where the leaching of the soil by percolating waters is above normal.

The foregoing evidence on superphosphate may be insufficient to provide rigid proof that the facts are best in harmony with the solution theory; but, on the other hand, no experimental work on superphosphates has, thus far, furnished any support for the theory of the absorption of colloids. If rigid proof were forthcoming that silica gels or sols are directly absorbed by plants, then, since it forms the greater portion of the outer coating of the soil colloidal particles, phosphorus and other ions absorbed by this hydrogel might be conceived to be carried into the root hairs with the silica, but until additional facts are obtained the view must be held to be purely speculative.

(2) *Sparingly soluble phosphates.* Is it valid to deduce from the fact that fertilizers, like basic slags and mineral phosphates, are "sparingly" soluble that solubility is not the *dominating* condition of availability, as Comber (14) suggests? The terms "sparingly soluble" and "valuable" applied to such fertilizers are purely relative. On some soils and for some plants, especially the *solanaceae*, as will be discussed in a subsequent paper, the mineral phosphates may be quite unavailable. Under favorable conditions, however, in some soils the mineral phosphates, especially "floats" (raw rock phosphate ground finer than 200 mesh), may be as available as the superphosphates.

(*a*) *Solubility and availability of "floats."* Due to their variable content of $CaCO_3$ there is considerable variation in the solubility of the natural rock phosphates in water, carbonic and citric acids, etc. The extensive bibliography on the subject is recorded by Cameron and Hurst (11). These latter investigators arrived at the conclusion that rock phosphate undergoes hydrolysis as follows:

$$Ca_3(PO_4)_2 + 4H_2O = CaHPO_4 + 2CaO \cdot H_2O + H_3PO_4$$

The "solvation" of this mineral by water is, therefore, a solution not of the tertiary phosphate but of its decomposition products. It is then these secondary products that are subjected to the action of the solvent forces of the soil solution. Moreover, the solubility of these products is related to their availability. Thus, Vanstone (88) has found that all the phosphates of calcium examined by him were practically completely soluble in 2 per cent oxalic acid in half an hour at ordinary temperatures and that the intake of phosphorus by the bean plant is proportional to the oxalic acid solubility.

(*b*) *Acids as a factor.* There is some evidence to indicate that acids, especially carbon dioxide, resulting from the decomposition of organic matter may, under some soil conditions, be an important factor. The laboratory experiments of Truog (86) and the field and laboratory work of Breazeale and Burgess (6) may be cited in this connection. The latter investigators found that in the case of wheat plants the relative absorption of PO_4 from rock phosphate in soil culture solutions, treated with "floats" contained in distilled water only, was only 0.0104 gm. PO_4, but 0.0188 gm. PO_4 when the solutions were saturated with CO_2 and 0.0206 gm. PO_4 from a dilute aqueous solution of $Ca(H_2PO_4)_2$. The availability of the rock phosphate used by these investigators in soil saturated with CO_2, therefore, is not much less than that of superphosphate. Although, as will be shown in a later paper, it is unlikely that the soil solution is saturated with CO_2, nevertheless, barring secondary reactions, the marked influence of this solvent on the availability of "floats" is indicated. It is probable that the reason why the earlier investigators failed to determine the influence of the CO_2 factor on the *availability* of rock phosphate was due not only to the reasons cited by Truog (86) and to reasons mentioned later (p. 263), but also to the unfortunate selection of the solvent used in extracting the phosphoric acid. When the extraction is carried out by means of the acids (citric, nitric) used by the earlier investigators, a reverse reaction sets in between the soluble calcium salt formed and the phosphoric acid that has become soluble, resulting in the precipitation of the phosphate (88).

From the foregoing it follows that an exact formula for the solubility of rock phosphate is not possible. It may be signified by the expression $s = \int \frac{H^+}{Ca^{++}}$, indicating that the solubility increases with the hydrogen-ion concentration of the solution. We should expect, therefore, as indeed Gedroiz (24) and McCall (56) and others have already shown, an increased availability of these naturally occurring phosphates on unsaturated soils. It has already been mentioned that Vanstone (88) found that the solubility of rock phosphate in 2 per cent oxalic acid corresponded with the rate at which mineral phosphates are absorbed by plants and, furthermore, Kelly's (47) laboratory experiments have indicated a depression in the solubility of $Ca_3(PO_4)_2$ in the presence of $CaCO_3$.

However, the solution of the problem of the availability of rock phosphates

to plants is not so simple as might be inferred from solubility results. The favorable factors leading to the solution of rock phosphate in the soil may be opposed by secondary reactions that tend to the "fixation" by bacteria (85) and to the precipitation by the hydrolyzing sol exchange bases, e.g., Al, Fe, (79) of the phosphorus thus rendered soluble. The new series of fertilizer plot experiments of the Pennsylvania Experiment Station, which were commenced in 1920, may be cited in illustration. In these experiments the additions of superphosphate and rock phosphate, respectively, to the *lime with manure* plots (pH 6.8) have given only approximately 15 per cent higher yields of corn, oats, and wheat than on the *lime without manure* plots (pH 5.9 to 6.0).

Current explanations, therefore, relative to the results obtained on the neutral or slightly acid soils of some of the eastern and middle western experiment stations, supplied with sufficient rainfall, to the effect that the amount of decomposing organic matter is the most important factor conditioning availability of rock phosphate, require qualification. Generalization of the explanations presented by Breazeale and Burgess (6) of their results obtained on the Western alkaline soils which they maintain contain no free CO_2 and which give no response to rock phosphate additions is, therefore, limited by other factors.

In many cases conclusions as to the relative availability of rock phosphate (and superphosphate) have been based on growth in sand culture experiments using mineral nutrient solutions. Such experiments, obviously, do not show the relative availabilities under field conditions.

There is evidence that the mineral acids and possibly also acetic, butyric and lactic acids formed in the soil increase the availability of sparingly soluble phosphates. This is supported by the work of Hopkins and Whiting (40) on the availability of rock phosphates in the presence of ammonium salts, and also by the Lipman process (53, 54) of rendering floats available by utilizing the oxidation of sulfur by micro-organisms.

(*c*) *Absorption of* PO_4 *from very low concentrations.* The concentration of phosphate ions in all soils is low. Numerous investigators, working with culture solutions, have shown that plants can develop normally in very dilute solutions of PO_4. Thus, Breazeale and Burgess (6) found that oat and barley seedlings can absorb PO_4 sufficient for normal development in a nutrient solution containing only 0.50 p.p.m.; and Parker (70) found that corn willl make maximum growth in culture solution containing only 0.25 p.p.m. PO_4. If these results are applicable to field conditions, the small solubility of rock phosphate would present no difficulties to the acceptance of the older solution theories.

Comber (15) points out that the naturally occurring phosphates do not seem to possess colloidal surfaces. But even if such a phosphate as "floats" was prepared in the colloidal condition and applied to the soil, it is questionable if carbon dioxide and the other solvents present would react with the colloid, owing to the influence of the electric charge carried by it.

The evidence presented affords no indication that the availability of rock phosphates is determined by any colloidal behavior. Rather does it point toward the conclusion that its availability is a question of absorption from solution as determined by the laws of diffusion and mass action, through the operation of which the rate of absorption of PO_4 ions by the plant is a function of the rate of solution of the mineral phosphate.

The behavior of other minerals is similar. Thus, the potassium or orthoclase has a solubility of 282 p.p.m. in distilled water. One could hardly, therefore, attribute the results obtained by Haley (32), in which 200-mesh orthoclase used in sand cultures furnished potassium at a rate ample to produce large yields of buckwheat, to the colloidal condition of the orthoclase.

(*d*) *Iron and aluminum phosphates.* Although there is evidence (58) to indicate that the *artificially prepared* phosphatic salts of iron and aluminum are more available to plants than the *naturally occurring* phosphates of these metals, there is nothing to indicate that colloidal behavior is the cause of this difference. Unfortunately, Marais (58) does not give the source of his "C.P." materials, nor the relative fineness of division of any of the phosphates used. Although it is possible that the greater fineness of division of the artificial phosphates as well as the tendency towards increasing basicity and consequent insolubility of the natural products, as weathering proceeds, may be contributary causes, other factors may predominate. Thus, in the case of artificially prepared ferric phosphate, for example, Cameron and Bell (10) have shown that the acidic properties of the salt prepared by precipitation may be very pronounced, even after 100 washings, due to absorbed PO_4 ions. The salt thus prepared is more soluble and, therefore, would be more available than the pure product.

Moreover, since Marais (58) did not find the *mineral* aluminum and iron phosphates of any value except in the presence of ammonium compounds, it would be logical to infer that the availability of these mineral phosphates in the presence of nitrogenous salts would be due to the action of the nitric acid formed (40), the rate of solution being determined by the number of particles below the critical stage (p. 260). However, it must be admitted that our knowledge of the conditions rendering the various phosphates in the soil soluble are very unsatisfactory. Over twenty years ago it was suggested by Whitson and Stoddart (94) that the reason why acid soils act as if deficient in phosphorus is due to the formation of aluminum (and iron) salts. Vanstone's (89) experiments on the solubility of soils in citric acid give confirmative evidence of the relative insolubility of the phosphorus of acid soils. But the findings of some investigators (58, 93) that the availability and, therefore, presumably the solubility of aluminum phosphate is increased by liming is not explicable on theoretical grounds. The equilibrium conditions of a solution involving Al (or Fe), Ca, PO_4, H and OH ions may be written

$$AlPO_4 \rightleftarrows Al^{+++} + PO_4^{---} \quad (1)$$
$$3Ca(OH)_2 \rightleftarrows 6OH^- + 3Ca^{++} \quad (2)$$

$$Al^{+++} + 6OH^- \rightleftarrows Al(OH)_3 \quad (3)$$
$$PO_4^{---} + 3Ca^{++} \rightleftarrows Ca_3(PO_4)_2 \quad (4)$$

As the solubility product of $AlPO_4$ is less than $Ca_3(PO_4)_2$, most of the PO_4 ions, at pH 7.0 and above, would be precipitated as $AlPO_4$ (or $FePO_4$). The tendency of lime would, therefore, be to depress the solubility of aluminum and ferric phosphates.

Teakle (81) suggests from the results of his experiments that the following reactions may take place.

$$AlPO_4 + 6OH^- \underset{\text{below pH } 6.4}{\overset{\text{above pH } 6.4}{\rightleftarrows}} AlO_3 + PO_4^- + 3H_2O \quad (1)$$

and

$$FePO_4 + 3OH^- \underset{\text{below pH } 3.0}{\overset{\text{above pH } 3.0}{\rightleftarrows}} Fe(OH)_3 + PO_4^- \quad (2)$$

also

$$Ca_3(PO_4)_2 + 6OH^- \xleftarrow{\text{neutral conditions}} Ca(OH)_2 + 2PO_4^- \quad (3)$$

$$x + \frac{y}{3} Ca_3(PO_4)_2 + 2y(OH)^- \xrightarrow[\text{alkaline conditions}]{} xCa_3(PO_4)_2 \cdot yCa(OH)_2 + \frac{2y}{3} PO_4^- \quad (4)$$

Under more alkaline conditions the calcic phosphate would give rise to a basic phosphate and PO_4 ions. But considerations of the solubility products of these substances lead to difficulties in accepting Teakle's conclusions *in toto*.

Austin (1) has challenged the belief that the formation of insoluble complexes of iron and aluminum phosphate in acid soils is the cause of the low availability of phosphorus found in many acid soils. He did not, however, as the title would seem to suggest, use any soils in his experiments. The data was obtained by working with the pure hydrosol or hydrogel bases assumed to be present in soils, by titrating electrometrically with $CaH_4(PO_4)_2$. Austin's conclusion is based on the fact that the titration of $Al(OH)_3$ and $Fe(OH)_3$ with $CaH_4(PO_4)_2$ resulted in 82 per cent and 40 per cent, respectively, of the phosphorus still being in solution at pH 5.4, from which fact he concludes that at pH 5.4 the equations

$$Al(OH)_3 + CaH_4(PO_4)_2 \rightarrow AlPO_4 + CaHPO_4 + 3H_2O$$
$$Fe(OH)_3 + CaH_4(PO_4)_2 \rightarrow FePO_4 + CaHPO_4 + 3H_2O$$

do not go to completion. In other words, in the presence of Al (or Fe), Ca, PO_4, H and OH ions a fairly acid soil would not have all the PO_4 ions combined with Al (or Fe).

The actual conditions in the soil are undoubtedly complex. Hydrous alumina has an isoelectric point of 6.5. If we consider its acidic dissociation

$Al(OH)_3 \rightarrow Al^{\cdots} + 3OH^-$ obviously the addition of an acid will result in the formation of water by the combination of the $H^{\cdot}$ of the acid and OH^- of the alumina. It follows, as Hardy (34) has indicated, that the colloidal hydrous alumina would be removed further from its isoelectric point and the potential difference between its surface and the dispersion medium would be increased. The addition of an anion, such as PO_4 or SiO_2, would—depending on the other anions and cations present—result in the formation of insoluble complexes. The precipitating power of other anions would depend on their relative electronegative affinity. As the soil solution became more acid we should expect Al (and Fe) ions to come into solution, for both aluminum phosphate and ferric phosphate become more soluble the higher the hydrogen-ion concentration of the medium. The system resulting would depend on the solubility products of the substances formed.

(*e*) *Basic slags.* The expression "insoluble but available to plants" by which clays have been characterized does not, without qualification, appear justifiable. The old Thomas slag obtained by the Thomas-Gilchrist-Bessemer process, which was produced by the addition of a basic "liming" of $CaCO_3$ and $MgCO_3$, was of a fairly constant composition, the phosphorus of which is from 80 to 95 per cent available to plants and showed close correlation with solubility in 2 per cent citric acid. The first "run off" used for the agricultural slag has a high phosphorus content. This Gilchrist-Bessemer process, however, has now, for economical reasons, been largely superceded in Europe by the Siemens-Martin process, in which a calcium fluoride flux is used, resulting in slags of very variable composition and phosphorus availability (6 to 50 per cent), but which show little correlation with solubility in 2 per cent citric acid. The slags from the acid-Bessemer and Basic Open Hearth processes used in the United States are not high in phosphorus and, moreover, the ores used for the making of steel in this country are invariably low in phosphorus.

Stead, as recorded by Vanstone (90), gives $Ca_3(PO_4)_2 \cdot CaO \cdot CaSiO_3$ as the composition of the low acid soluble slags. The most soluble phosphate has the composition $5CaO \cdot SiO_2 \cdot P_2O_5$. When these silico-phosphates are applied to the soil the combined CaO is freed as weathering proceeds, permitting the ready dissolution of the P_2O_5 radical. The period of time for this weathering process to occur and, therefore, the availability varies with the nature of the slag. The smaller the amount of silicate in the slag the higher the availability.

The behavior of slags in the soil, therefore, does not tend, without further experimental evidence, to support any theory of colloidal absorption by plants.

ACKNOWLEDGMENT

The author takes this opportunity of expressing his appreciation for the many suggestions and criticisms relating to the manuscript received from Dr. G. C. Chandlee.

REFERENCES

(1) AUSTIN, R. H. 1927 Some reactions between mono-calcium phosphate and soils. *Soil Sci.* 24: 263–269.

(2) BASSETT, H. 1917 The phosphates of calcium. IV. The basic phosphates. *Jour. Chem. Soc.* (London) 111: 620–642.

(3) BEANS, H. T., AND EASTLACK, HERBERT E. 1915 The electrical synthesis of colloids. *Jour. Amer. Chem. Soc.* 37: 2667–2683.

(4) BECHHOLD, H. 1919 Colloids in Biology and Medicine. New York.

(5) BLAIR, A. W., AND PRINCE, A. L. 1924 Some changes brought about in cylinder soils by long continued crop and fertilizer treatment. *Soil Sci.* 18: 31–52.

(6) BREAZEALE, J. F., AND BURGESS, P. S. 1926 The availability of phosphates in calcareous or alkaline soils. *Ariz. Agr. Exp. Sta. Tech. Bul.* 10: 209–237.

(7) BROWN, WILLIAM 1915 On the preparation of collodion membranes of differential permeability. *Biochem. Jour.* 9: 591–617.

(8) BROWN, WILLIAM 1917 Further contributions to the technique of preparing membranes for dialysis. *Biochem. Jour.* 11: 40–57.

(9) BUTKEWITSCH, W. S., AND BUTKEWITSCH, W. W. 1925 Zur Frage nach der Rolle des "Donnanschen Membrangleichgewichts" bei osmotischen Vorgängen im lebenden Zellen. *Biochem. Ztschr.* 161: 468–487.

(10) CAMERON, F. K., AND BELL, J. M. 1907 The action of water and aqueous solutions upon soil phosphates. U. S. Dept. Agr. Bur. Soils Bul. 41.

(11) CAMERON, F. K., AND HURST, L. A. 1904 The action of water and saline solutions upon certain slightly soluble phosphates. *Jour. Amer. Chem. Soc.* 26: 885–913.

(12) CAMERON, F. K., AND ROBINSON, W. O. 1908 The action of carbon dioxide under pressure upon a few metal hydroxides at 0°C. *Jour. Phys. Chem.* 12: 561–573.

(13) CLOWES, G. H. A. 1916 Protoplasmic equilibrium. I. Action of antagonistic electrolytes on emulsions and living cells. *Jour. Phys. Chem.* 20: 407–451.

(14) COMBER, NORMAN M. 1922 The availability of mineral plant food. A modification of the present hypothesis. *Jour. Agr. Sci.* 12: 363–369.

(15) COMBER, NORMAN M. 1922 The flocculation of soils. III. *Jour. Agr. Sci.* 12: 372–386.

(16) COMBER, NORMAN M. 1924 The rôle of electronegative ions in the reactions between soils and electrolytes. Faraday Soc. Gen. Discuss. 567–572.

(17) COMBER, NORMAN M. Private communication.

(18) CURTIS, OTIS F. 1920 The upward translocation of foods in plants. I. Tissues concerned in translocation. *Amer. Jour. Bot.* 7: 101–124.

(19) CURTIS, OTIS F. 1926 What is the significance of transpiration? *Science* (n.s.) 63: 267–271.

(20) CZAPEK, F. 1920 Biochemie der Pflanzen, vol. 1, p. 62. Jena.

(21) DENISON, IRVING A. 1922 The nature of certain aluminum salts in the soil and their influence on ammonification and nitrification. *Soil Sci.* 13: 81–106.

(22) DHÉRÉ, C. 1927 Die Elektrodialyse in der Biochemie. *Kolloid. Ztschr.* 4: 243–258; 315–326.

(23) GAEDERTZ, A., AND WITTGENSTEIN, A. 1928 Über die Permeabilität der Blut—Kammerwasser—Scheide. Experimentalle Untersuchungen über den Stofftransport von Blut ins Kammerwasser. *Klin. Wchnchr.* 7: 63–65.

(24) GEDROIZ, K. K. 1911 The action of phosphates on soils saturated and unsaturated with bases. *Russ. Jour. Expt. Landw.* 12: 529–546.

(25) GILE, P. L., AND CARRERO, J. O. 1915 Assimilation of colloidal iron by rice. *Jour. Agr. Res.* 3: 205–210.

(26) Gile, P. L., and Carrero, J. O. 1920 Cause of lime-induced chlorosis and availability of iron in the soil. *Jour. Agr. Res.* 20: 33–61.
(27) Gile, P. L., and Smith, J. G. 1925 Colloidal silica and the efficiency of phosphates. *Jour. Agr. Res.* 31: 247–260.
(28) Gordon, Neil E., and Starkey, E. B. 1922 Influence of soil colloids on availability of salts. *Soil Sci.* 14: 1–7.
(29) Graham, Thomas 1862 Liquid diffusion applied to analysis. *Phil. Mag. and Jour, Sci.* (4) 23: 204–223.
(30) Gregoire, A. 1911 L'action sur les végétaux superieur de quelques sels hydrolysables et de quelques colloides. *Bul. Soc. Chem. Belg.* 25: 85–103.
(31) Gurchot, Charles 1926 Reversible permeability of membranes and its relation to cell metabolism. *Jour. Phys. Chem.* 30: 83–105.
(32) Haley, D. E. 1922 The availability of potassium in orthoclase in plant nutrition. *Soil Sci.* 15: 167–180.
(33) Hall, A. D. 1912 The Soil, p. 157. New York.
(34) Hardy, F. 1926 Anomalous flocculation in colloidal clays and soils. *Jour. Phys. Chem.* 30: 254–256.
(35) Hardy, F. 1926 The rôle of aluminum in soil infertility and toxicity. (A review.) *Jour. Agr. Res.* 16: 616–631.
(36) Harrer, Norman J. 1929 Study of solvent effects of certain organic acids upon oxides of iron as related to the iron cycle in nature. *Jour. Econ. Geol.* 24: 50–61.
(37) Hoagland, D. R. 1923 The absorption of ions by plants. *Soil Sci.* 16: 225–246.
(38) Höber, R. 1909 Die Durchlassigkeit der Zellen für Farbstoffe. *Biochem. Ztschr.* 20: 56–99.
(39) Höber, R. 1922 Physikalische Chemie der Zelle und der Gewebe, p. 367 et seq., 5 ed. Leipzig.
(40) Hopkins, Cyril G., and Whiting, Albert L. 1916 Soil bacteria and phosphates. *Ill. Agr. Exp. Sta. Bul.* 190: 395–406.
(41) Jacobs, M. H. 1924 Article on "Permeability of the cell to diffusing substances" in Cowdry's General Cytology, 99–164. Univ. of Chicago Press.
(42) Jennings, David S. 1919 The effect of certain colloidal substances on the growth of wheat seedlings. *Soil Sci.* 7: 201–215.
(43) Joffe, J. S., and McLean, H. C. 1928 Colloidal behavior of soils and soil fertility. IV. Anion effect on the precipitation reactions and degree of dispersion of aluminum and iron hydroxides. *Soil Sci.* 26: 47–59.
(44) Joseph, A. F., and Hancock, J. S. 1923 The action of silica on electrolytes. *Jour. Chem. Soc.* 123: 2022–2025.
(45) Kahlenberg, Louis 1906 On the nature of the process of osmosis and osmotic pressure with observations concerning dialysis. *Jour. Phys. Chem.* 10: 141–209.
(46) Kahlenberg, Louis 1926 On the separation of crystalloids from one another by dialysis. *Phil. Mag. and Jour. Sci.*, (7) 1: 385–394.
(47) Kelley, W. P. 1918 Effect of nitrifying bacteria on the solubility of tricalcium phosphate. *Jour. Agr. Res.* 12: 671–683.
(48) Lemmermann. O., Hasse, P., and Jessen, W. 1928 Die Beziehungen zwischen Pflanzenernährung und Pflanzenwachstum und die Methode Mitscherlichs zur Bestimmung des Düngerbedürfnisses des Bodens. *Ztschr. Pflanzenernähr. u. Düngung*, B7: 49–98.
(49) Lemmermann, O., and Wiesmann, H. 1922 Die ertragssteigernde wirkung der Kieselsäure bei unzureichender Phosphor-säureernährung der Pflanzen. *Ztschr. Pflanzenernähr. u. Düngung*, A1: 185–255.
(50) Lenher, Victor, and Merrill, Henry Baldwin. 1917 The solubility of silica. *Jour. Amer. Chem. Soc.* 39: 2630–2638.
(51) Lewis, Gilbert N. 1916 The atom and the molecule. *Jour. Amer. Chem. Soc.* 38: 762–785.

(52) LIPMAN, C. B. 1918 A new method of extracting the soil solution. *Univ. Calif. Pub. Agr. Sci.* 3: 131–134.

(53) LIPMAN, JACOB G., MCLEAN, HARRY C., AND LINT, H. CLAY 1916 Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *Soil Sci.* 2: 499–538.

(54) LIPMAN, JACOB G., AND MCLEAN, HARRY C. 1918 Experiments with sulfur-phosphate composts conducted under field conditions. *Soil Sci.* 5: 243–250.

(55) LOEB, J. 1924 "Crystalloid and colloidal behavior of proteins" in Bogue's Theory and Application of Colloidal Behavior, p. 23. New York.

(56) MCCALL, A. G. 1926 The relative value of different carriers of phosphorus. *Md. Agr. Exp. Sta. Bul.* 289: 155–178.

(57) MCGEORGE, W. T. 1924 Iron, aluminum and manganese in the soil solution of Hawaiian soils. *Soil Sci.* 18: 1–11.

(58) MARAIS, JACOBUS STEPHANUS. 1922 The comparative agricultural value of insoluble mineral phosphates of aluminum, iron and calcium. *Soil Sci.* 13: 355–409.

(59) MICHAELIS, L., AND WEECH, A. A. 1928 Studies on the permeability of membranes. IV. Variations of transfer numbers with the dried collodion membrane produced by the electric current. *Jour. Gen. Physiol.* 11: 147–158.

(60) MITSCHERLICH, A. 1925 Die Bestimmung des Düngerbedürfnisses des Bodens. Berlin.

(61) MUENSCHER, W. C. 1922 Effect of transpiration on absorption of salt by plants. *Amer. Jour. Bot.* 9: 311–329.

(62) MURRAY, J. ALAN 1924 The available state. *Soil Sci.* 17: 359–371.

(63) MYLIUS, F., AND GROSEHUFF, E. 1906 α und β-Kieselsäure in Losung. *Ber. Deut. Chem. Gesell.* 39: 116–125.

(64) NORTON, T. H., AND ROTH, D. M. 1897 On the existence of orthosilicic acid. *Jour. Amer. Chem. Soc.* 19: 832–835.

(65) OAKLEY, HENRY BOWEN 1926 The origin of the charge on colloidal particles. *Jour. Phys. Chem.* 30: 902–916.

(66) OSTERHOUT, W. J. V. 1917 Does the temperature coefficient of permeability indicate that it is chemical in nature? *Bot. Gaz.* 63: 317–320.

(67) OVERTON, E. 1899 Über die allgemeine osmotische Eigenschaften der Zelle. *Vierteliahrb. Naturforscherges Zurich* 44: 889.

(68) OVERTON, E. 1902 Beitrag zur allgemeine Muskel-und nervenphysiologie. *Arch. f. d. ges. Physiol.* 92: 115–280.

(69) PARKER, F. W. 1921 Methods of studying the concentration and composition of the soil solution. *Soil Sci.* 12: 209–232.

(70) PARKER, F. W. 1927 Soil phosphorus studies. III. Plant growth and the absorption of phosphorus from culture solutions of different phosphate concentrations. *Soil Sci.* 24: 129–146.

(71) PFEFFER, T. 1900 Physiology of plants, Vol. I, p. 435. Translated by A. J. Ewart. Oxford.

(72) PRÁT, SILVESTR 1923 Die Elektrolytaufnahme durch die Pflanze. I. Die Resorption von Mineralstoffen durch die Wurzeln. *Biochem. Ztschr.* 136: 366–376.

(73) RAMANN, E. 1916 Über Mineralstoffaufnahme der Pflanzen aus dem Boden. *Die Landw. Vers. Sta.* 88: 379–395.

(74) RHINE, J. B. 1926 Translocation of fats as such in germinating fatty seeds. *Bot. Gaz.* 82: 154–164.

(75) ROBINSON, G. W., AND JONES, J. O. 1927 Losses of added phosphate by leaching from North Welsh soils. *Jour. Agr. Sci.* 17: 94–103.

(76) RUHLAND, W. 1912 Studien über die Aufnahme von Kolloiden durch die Pflanzliche Plasmahaut. *Jahrb. Wiss. Bot.* 51: 376–431.

(77) SCHMIDT, R. H. 1891 Über Aufnahme und Verarbeitung von fetten Ölen durch Pflanzen. *Flora* 74: 300–370.

(78) SORUM, C. HARVYY 1928 The preparation of chloride free colloidal ferric oxide from ferric chloride. *Jour. Amer. Chem. Soc.* 50: 1263–1267.

(79) SPURWAY, C. H. 1925 Some factors influencing the solubility of phosphorus in soil-acid phosphate mixtures. *Soil Sci.* 19: 399–405.

(80) STARKEY, E. B., AND GORDON, NEIL E. 1922 Influence of hydrogen-ion concentration on the adsorption of plant food by soil colloids. *Soil Sci.* 14: 449–457.

(81) TEAKLE, L. J. H. 1928 Phosphate in the soil solution as affected by reaction and cation concentrations. *Soil Sci.* 25: 143–162.

(82) THOMAS, ARTHUR W. 1925 The modern trend in colloid chemistry. *Jour. Chem. Educ.* 2: 323–340.

(83) THOMAS, ARTHUR W., AND FRIEDEN, ALEXANDER 1923 Ferric salt as the "solution link" in the stability of ferric oxide hydrosol. *Jour. Amer. Chem. Soc.* 45: 2522–2532.

(84) THOMAS, WALTER 1919–21 Soil solution investigations. Unpublished.

(85) TOTTINGHAM, W. E., AND HOFFMAN, C. 1913 Nature of the changes in the solubility and availability of phosphorus in fermenting mixtures. *Wisconsin Agr. Exp. Sta. Res. Bul.* 29: 273–321.

(86) TRUOG, E. 1912 Factors influencing the availability of rock phosphate. *Wisconsin Agr. Exp. Sta. Res. Bul.* 20: 17–51.

(87) TRUOG, E. 1925 The significance of soil colloids in relation to plant feeding and conservation of essential elements. *Jour. Amer. Soc. Agron.* 17: 280–285.

(88) VANSTONE, ERNEST 1925 Basic slags and mineral phosphates. *Jour. Agr. Sci.* 15: 36–46.

(89) VANSTONE, ERNEST 1925 The available phosphate in soils. *Jour. Agr. Sci.* 15· 460–465.

(90) VANSTONE, ERNEST 1927 The chemistry of basic slags. *Jour. Agr. Sci.* 17: 143–152.

(91) WARINGTON, R. 1873 On the decomposition of tricalcic phosphate by water. *Jour. Chem. Soc.* (London) 26: 983–989.

(92) WEECH, A. A., AND MICHAELIS, L. 1928 Studies on permeability of membranes. V. The diffusion of non-electrolytes through the dried collodion membrane. *Jour. Gen. Physiol.* 12: 55–81.

(93) WHEELER, H. J., AND ADAMS, G. E. 1906–07 A comparison of nine different phosphates upon limed and unlimed land with several varieties of plants. *Rhode Island Agr. Exp. Sta. Buls.* 114: 117–137; 118: 55–86.

(94) WHITSON, A. R., AND STODDART, C. W. 1906 Availability of phosphates in relation to soil acidity. I. Soil acidity as an indication of lack of available phosphates. *Wisconsin Agr. Exp. Sta. 23rd. Ann. Rpt.*: 171–180.

(95) WILLIAMS, E. C. 1924 Silica gel as an industrial absorbent. *Jour. Soc. Chem. Indus.* 43: 97T–112T.

(96) WILSON, JOHN ARTHUR 1916 Theory of colloids. *Jour. Amer. Chem. Soc.* 38: 1982–1985.

(97) WITZEMANN, EDGAR J. 1924 Leucoplasts: Living reproducing, perfect chemical catalysts. *Jour. Phys. Chem.* 28: 305–312.

(98) WOLKOFF, M. I. 1924 Relative availability of the phosphorus of raw rock and acid phosphate in soils. *Soil Sci.* 17: 39–56.

CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: III. CHEMICAL STUDIES OF TWO FLORIDA PEAT PROFILES

SELMAN A. WAKSMAN AND KENNETH R. STEVENS

New Jersey Agricultural Experiment Station

Received for publication January 24, 1929

In a previous contribution (10), the authors suggested a system of proximate analysis of peat material which accounts for about 90 per cent of its constituents in the form of definite chemical complexes. This system of analysis has been devised with the idea of throwing light upon the chemistry of the organic constituents of peat and upon the chemical processes involved in their formation from the natural plant materials. It was found that in all the lowmoor and sedimentary peats examined, the celluloses are completely or almost completely decomposed while some of the hemicelluloses, largely of a hexosan nature, are left; the highmoor peats, however, were found to contain considerable quantities of both celluloses and hemicelluloses. This marked difference in the chemical composition of these types of peat was found to be due not only to the specific environmental conditions prevailing in the peat bogs during plant growth and decomposition, but largely to the chemical nature of the organic constituents of those plant associations which gave origin to the specific peat formations.

Lowmoor and sedimentary peats are also known to contain large quantities of organic nitrogenous complexes, the nitrogen content of these specific types of peat being considerably higher than that of the plants from which they have originated; on the other hand, the highmoor peats contain only a limited amount of organic nitrogen compounds, frequently even less than the plants from which these peats have originated. These important differences were explained as due largely to the nature of the decomposition processes of the various plant constituents, brought about by the microörganisms which are active in the formation of the different types of peat. The highmoor peats contain a considerably larger amount of fatty and waxy substances than the lowmoor and sedimentary peats. The low mineral content and high acidity of the highmoor peats, the fairly high mineral content and limited acidity of the lowmoor peats, and the great abundance to almost predominance of mineral matter and neutrality to alkalinity of the sedimentary peats are also characteristic phenomena for differentiating the chemical composition of these important formations of peat.

The following studies deal with two more profiles, one lowmoor and one sedimentary, which were taken from a distinctly different region and which result from different plant associations and are formed under conditions

entirely different from those of the New Jersey peat profiles. These studies were undertaken with the idea of throwing further light upon the chemical composition of peat and upon the chemical and biological processes involved in its formation from natural vegetation.

The lowmoor peat used in these investigations represents a typical profile of the Everglade peat area in Florida. The authors are indebted to Dr. R. V. Allison of the Everglade substation at Belle Glade, for supplying the necessary material. The profile was taken about four miles south of Lake Okeechobee and about a quarter mile west of Okeelanta.

The sedimentary profile was taken from a typical allochtonous peat formation, referred to by European investigators as Gyttja. This peat has been formed in standing water, in a lake known as "Mud Lake," about 20 miles northeast of Ocala in Northern Florida and about 80 miles SSW of Jacksonville, Florida. The lake is fed by a series of springs and is bordered on the southwest and north by swamps, and on the east by a wooded hill; it drains on the northwest side into a river by means of a small creek. At the time of sampling, the surface of the peat was about three feet below the surface of the lake, while its depth extends to about forty-five feet. The authors are indebted to the Cummer Lumber Co. for supplying the required material from this peat formation.

The Everglade lowmoor, or saw grass, profile was divided into seven sections, designated as follows:

Number of sample	*Depth cm.*	
50	0–7.5	Dark brown to black, finely decomposed surface material with varying quantities of undecomposed fiber
51	7.5–30	Upper fibrous layer (*Cladium*) gradually grading into pure Cladium peat
52	30–45	Layer of dense fibrous peat derived entirely from saw grass (*Cladium*)
53	45–55	Lower depth of fibrous layer and adjoining black compact layer of sedimentary peat
54	55–70	Central portion of sedimentary layer consisting of black, compact, colloidal material, with a minimum of fibrous material
55	75–120	Lower portion of sedimentary layer with adjoining upper portion of lower fibrous layer
56	125–135	Lower fibrous layer of *Cladium* peat

At a depth of 185 cm. rock formation is underlying the peat area.

The fibrous layer consists of practically pure saw grass or *Cladium*. The sedimentary or plastic layer is a typical allochtonous formation resulting from an accumulation of wind blown, sedimentary vegetable material as well as of various water plants formed in permanent quiet bodies of water. When the lake-formed deposits have been built up to a point less than 10 feet below the average water level, species of *Nymphaeaceae*, *Cladium*, *Sagittaria*, etc., appear which gradually give rise to the brown fibrous peat which is autochtonous in nature. This peat is later invaded by lowland trees. According to Forsaith (4), the allochtonous peat contains calcareous remains of *Chara*,

diatomaceous tests, limy silts, etc.; pollen grains of Abietineae and catkin-bearing angiosperms, spores of ferns, fungi; strips of cutinized epidermis, idioblasts of water lilies and fragments of woody and herbaceous plants; animal derivatives, such as chitinized portions of insects, spicules of fresh water fungi, infusoria, shells of mollusks and protozoa. This mass of material can be distinguished even from the well-decomposed plant residues of autochtonous peat (when the water level was low), both by microscopic structure and by chemical composition.

Dachnowski (3) analyzed both the sedge peat and the sedimentary peat in approximately the same part of the Everglades from which the samples used in these investigations were obtained, with the following results: The air-dry sedge peat contained 11.24 per cent water, 7.2 per cent ash, 19.63 per cent protein and 0.25 per cent fat, whereas the sedimentary layer contained 9.81 per cent water, 32.67 per cent ash 15.75 per cent protein and 0.15 per cent fat. On an ash-free and moisture-free basis, the sedge peat contained 24.07 per cent protein and the sedimentary peat 27.38 per cent.

Miller (6) reported the following inorganic constituents in the plants of *Cladium eflusum*, on per cent basis of dry material: SiO_2 – 0.30; Fe_2O_3 + Al_2O_3 – 0.11, CaO – 0.57, MgO – 0.11, Na_2O – 0.16, K_2O – 0.35, P_2O_5 – 0.07, and nitrogen – 0.8. The saw grass peat contained, on a similar basis: total ash 6.8 to 7.7, SiO_2 – 1.95 to 2.04, Fe_2O_3 + Al_2O_3 – 0.60 to 0.69, CaO – 2.74 to 3.02, MgO – 0.44, Na_2O – 0.14 to 0.19, K_2O – 0.06 to 0.11, P_2O_5 – 0.13 to 0.15, nitrogen – 3.32 to 3.84 per cent. The results show that with the decomposition of the saw grass, there is a considerable loss of potassium and a gain in nitrogen and various bases. Assuming that there was no loss of silicon in the process of peat formation from the saw-grass plants, Miller calculated that about seven parts of plant material were required to produce one part of peat.

Rose (7) found 0.067 per cent K_2O in the saw grass peat and 0.122 per cent in the sedimentary peat. Hammar (5) made a detailed analysis of the inorganic constituents of several of the peat areas in the Everglades, namely the saw grass peat, the sedimentary peat itself which gives the so-called custard-apple soil or plastic soil, and an intermediary peat, which gives the so-called elderberry soil. His results can be summarized as follows, on a per cent basis of dry peat:

CONSTITUENTS	SAW-GRASS PEAT	SEDIMENTARY PEAT	INTERMEDIATE PEAT
	per cent	*per cent*	*per cent*
Organic matter	86.37	44.52	77.65
Nitrogen	2.79	1.47	2.65
Ash	13.63	55.48	20.35
SiO_2	4.12	35.81	9.87
Fe_2O_3	1.01	5.04	1.81
CaO	5.21	3.81	5.25
MgO	0.10	0.09	0.13
P_2O_5	0.42	0.48	0.42
Al_2O_3	0.30	5.08	0.78

The sedimentary peat profile was taken, in one foot sections, from the surface of the peat (3 feet below surface of lake) to a depth of 12 feet. These twelve sections were combined so as to give only 6 layers of the profile, including the upper one foot, 1–2, 2–4, 4–6, 6–8, 8–10, and 10–12 feet. In the analyses reported in the previous papers (11), only sedimentary layers were analyzed from lowmoor and highmoor peat profiles. Those layers were characterized by a high ash content, frequently as much as 60 per cent of the total dry material, a high protein content (10 to 24 per cent), practically no cellulose, varying amounts of hemicellulose and a low ether soluble fraction.

The chemical composition of the Everglade peat has attracted considerable attention, just as the whole problem of the utilization of the Everglades

TABLE 1

Chemical composition of Florida (Everglade) peat profile

On per cent basis of dry material

NUMBER OF SAMPLE	PEAT FORMATION	DEPTH OF LAYER	ASH	CRUDE PROTEIN	ETHER SOLUBLE	COLD AND HOT WATER SOLUBLE	ALCOHOL SOLUBLE	HEMICELLULOSE	CELLULOSE	LIGNIN	TOTAL
		cm.									
50	Surface layer	0–6.5	12.09	22.75	2.96	1.31	1.22	6.87	0.30	43.72	91.22
51	Upper fibrous layer	6.5–26	10.00	23.06	2.98	1.73	1.06	6.41	0.28	46.12	91.64
52	Typical saw-grass peat	26–40	6.86	22.25	3.16	1.60	1.31	8.04	0.43	44.90	88.55
53	Lower fibrous layer	40–50	8.05	21.25	3.21	1.45	1.39	7.63	0.43	47.63	91.04
54	Sedimentary layer	50–62	59.60	9.00	2.97	1.07	0.45	2.19	0	19.33	94.61
55	Lower portion of sedimentary layer	62–70	42.37	12.98	1.46	0.82	0.68	2.64	0	28.47	89.42
56	Lower fibrous layer	110–120	15.14	20.38	1.58	0.84	1.23	4.30	0	48.44	91.91

However, as seen from the previous references, very little attention has been paid to the chemical nature of the organic complexes in this most extensive peat formation, and to the influence that this may have upon the productivity of the peat when it is changed by draining and cultivation, into a crop-producing soil.

The seven horizons of the saw-grass peat profile were analyzed according to the method previously described. The results are presented in table 1. To enable us to compare the abundance of the various organic complexes in the several layers of the profile, the figures representing the hemicellulose, lignin and protein content of the different horizons are calculated on an ash-free, dry basis and reported in table 2.

The results of the chemical composition of this lowmoor peat profile confirm

the observations of other investigators in regard to the organic matter, ash and nitrogen content of the saw-grass and sedimentary peat formations. Not only the younger saw-grass peat or the layers above the sedimentary peat but also the older saw-grass peat, or the layer below the sedimentary peat contain about 93 to 85 per cent organic matter, while the sedimentary material itself is very high in inorganic matter and contains only 40.40 to 57.43 per cent of organic substances.

The upper horizons of the profile, namely the younger saw-grass peat, still contain small amounts of cellulose, while the sedimentary peat and the layers of older saw-grass peat are entirely free from cellulose. The hemicellulose content is also considerably higher in the younger saw-grass peat than in the sedimentary and the older fibrous peats. One must recall in this connection that the saw-grass plants were found to contain 28.3 per cent cellulose in the stems and 30.7 per cent in the roots, as well as 21.5 and 20.8 per cent pentosan in the stems and roots respectively. In other words, as a result

TABLE 2

Chemical composition of the organic matter of an Everglade peat profile

On per cent basis of moisture-free and ash-free material

NUMBER OF SAMPLE	HEMICELLULOSE	LIGNIN-LIKE COMPLEXES	PROTEIN
50	7.81	49.68	25.85
51	7.12	51.24	25.62
52	8.64	48.23	23.90
53	8.29	51.66	24.18
54	5.42	47.80	22.28
55	4.58	49.43	22.53
56	5.06	57.05	24.01

of decomposition of the plant residues by microörganisms in the process of peat formation, nearly all the cellulose and a large part of the hemicellulose have disappeared. It is again important to call attention to the fact pointed out previously (11) that while in the fresh saw-grass plants most of the hemicelluloses are made up of pentosans, in the peat itself most of the hemicellulose is of a hexosan nature. This points to as nearly complete a decomposition of the pentosans as of the celluloses and to an accumulation of certain hemicelluloses of a hexosan nature resistant to decomposition, some of which are of plant origin and some have no doubt been synthesized by the microorganisms bringing about the decomposition processes in the peat.

The protein content, determined by total nitrogen, is considerably higher in the saw grass than in the sedimentary peat, but the difference becomes less marked when calculated upon an ash-free basis. The same is true of the lignin-like complexes, or that fraction of the organic matter which is insoluble in ether, alcohol, boiling dilute hydrochloric acid and cold 80 per cent sulfuric acid, followed by boiling in 5 per cent of this acid. The lower or

older saw grass peat contains considerably more lignin and less of the ether and water soluble materials as well as of the hemicellulose fraction than the younger saw-grass layers of the peat profile. This, as well as the complete absence of cellulose, indicates that considerably greater decomposition has taken place in the older saw-grass peat than in the younger formations.

Definite chemical differences are thus found to exist not only between the saw-grass and sedimentary peats, but also between the younger and older saw-grass peat formations. It would be of considerable interest to determine whether these differences have an influence upon the rapidity of decomposition of peat and the liberation of the nutrients, especially of the nitrogen, in an available form. Fortunately, the sedimentary peat forms large outcroppings, where no surface layer of saw-grass peat has as yet been formed, namely in the form of a narrow zone just adjoining the lake Okeechobee. When drained and cultivated, the sedimentary peat, known locally as "custard-apple soil," is found to be very fertile, while the saw-grass peat is of considerably lower fertility as far as the growth of cultivated plants is concerned.

TABLE 3

Evolution of CO_2 and formation of nitrate from 100-gm. portions of different layers of an Everglade peat profile

NUMBER OF LAYER	TOTAL CO_2-EVOLUTION (MGM. OF C)			NITRATE FORMATION (MGM. N)	MOISTURE CONTENT (PER CENT)
	9 days incubation	18 days incubation	32 days incubation	32 days incubation	
50	15.0	24.7	41.8	13.24	66.8
52	8.3	13.5	24.9	12.5	71.4
53	7.0	10.8	18.2	5.5	88.3
54	2.5	4.0	6.5	5.1	85.0
55	3.6	6.3	12.0	1.6	75.8
56	3.8	7.4	12.9	0.6	83.4

Although a detailed survey of the microörganisms found in different layers of various peat profiles and of the microbiological processes taking place in the formation of peat and in the decomposition of peat when the latter is drained and cultivated, is reserved for a later publication, certain data will be presented here in an attempt to throw light upon any possible differences in the transformations which take place in the saw-grass and sedimentary peat regions in the Everglades when they are subject to the action of a mixed flora and fauna of microörganisms.

One-hundred-gram portions of the natural moist peat were taken from the various layers of the profile, placed in aeration flasks and incubated for 32 days, at 25°C. At the end of that period of time the total nitrate nitrogen was determined. The evolution of carbon dioxide from each 100-gram portion of moist peat and the liberation of nitrogen in the form of nitrate are reported in table 3.

The results show that there is a gradual decrease in the rapidity of liberation of carbon dioxide and formation of nitrate nitrogen with the depth of the peat profile. One must consider of course the fact that the moisture content of the material taken from the different layers varies, and, when calculated on the basis of actual amount of dry peat undergoing decomposition, different results will be obtained: 33.2 gm. of the dry peat of No. 50, or the uppermost layer, gave off, in 32 days, 41.8 mgm. of C as CO_2 and allowed the accumulation of 13.2 mgm. of nitrate nitrogen. An equal amount of peat in the form of No. 52 gave off 28.9 mgm. of C as CO_2 and produced 14.5 mgm. of nitrate nitrogen. The same amount of peat in horizon No. 53 gave off 51.7 mgm. of C as CO_2 and liberated 15.6 mgm. of nitrate nitrogen. An equal amount of peat in the form of No. 54, or the sedimentary layer, produced 14.4 mgm. of C as CO_2 and liberated 11.3 mgm. of nitrate nitrogen.

In other words, the seemingly marked differences would tend to disappear when compared on a dry basis of the peat undergoing decomposition. If a comparison is further made on the basis of ash-free peat, the results for the sedimentary peat (No. 54) would be further increased due to the high ash content of this horizon.

In general, one must conclude from these data that the rapidity of decomposition of the organic matter in the various peat layers is rather slow, as indicated by the slow evolution of carbon dioxide, when this is compared with the rapidity with which carbon dioxide is given off in the decomposition of fresh organic matter. However, in the decomposition of the peat there is rapid evolution of nitrogen in an available form. With the exception of the very lowest layers of older saw-grass peat, the ratio between the carbon liberated as carbon dioxide and the nitrogen formed as nitrate is very narrow, almost 1.3 to 1 in the case of sedimentary peat and 2–3.3 to 1 in the upper layers of saw-grass peat. In the case of ordinary soil organic matter the ratio of the carbon liberated as CO_2 to the nitrogen formed as nitrate is about 10:1, or very similar to the ratio of carbon to nitrogen in the soil organic matter itself (8).

These results tend to indicate that the nitrogenous complexes, in the Everglade peat at least, undergo much more rapid decomposition than the non-nitrogenous organic complexes. In other words the very definite ratio between the carbon to nitrogen has not been established as yet when compared with the organic matter in normal field soils. What will happen in these peats upon prolonged cultivation still remains to be shown. It is quite possible that the available nitrogen may be rapidly liberated and the carbon-nitrogen ratio actually widened. This may bring about later the actual need for nitrogen fertilization in an injudicious management of the soil. The economic problem might then become somewhat similar to that of the high-moor peats, where there was only little nitrogen to begin with.

To be able to compare the decomposition processes that would take place in these peat formations when brought to a lower moisture content, and to

have a more correct basis for comparison of the rapidity of decomposition of the organic matter in the different horizons, the various peat samples were partly air dried, then adjusted to the same moisture content; thus, 75 grams of material taken for the decomposition studies contained 25 grams of dry matter and 50 grams of water, giving a moisture content of 66.7 per cent. These preparations were placed in aeration flasks and incubated as before. Both ammonia and nitrates were determined in the peats at the end of the incubation period. The results reported in table 4 are quite similar to those obtained with the naturally moist peat. The younger saw-grass peat and surface layers have decomposed more rapidly than the lower layers, especially the older fibrous peat. These results confirmed the previous observations concerning the very narrow ratio between the carbon liberated as CO_2 and the nitrogen made available as ammonia and nitrate.

The rapid liberation of the nitrogen in the peat in an available form no doubt accounts for the high fertility of these bogs when they are drained and brought

TABLE 4

Evolution of CO_2 and formation of nitrate from different layers of an Everglade peat profile, using 25-gm. portions of dry material and 200 per cent moisture

NUMBER OF LAYER	TOTAL CO_2 EVOLUTION (MGM. C)				NITROGEN LIBERATION IN 32 DAYS (MGM.)		
	5 days incubation	12 days incubation	26 days incubation	32 days incubation	NH_3	NO_3-N	Total N
50	11.5	23.9	44.6	54.8	4.82	13.20	18.02
52	16.9	28.6	44.4	51.8	2.45	23.24	25.69
54	3.8	7.0	13.6	15.7	1.25	5.70	6.95
56	2.9	6.7	16.2	21.1	1.20	3.45	4.65

under cultivation. Although certain differences are found both in the chemical composition and in the rapidity of decomposition between the typical saw-grass peat and the sedimentary or plastic peats, these differences are not sufficient to explain why the latter type of peat (as found around Lake Okeechobee) should prove to be much more fertile than the saw-grass peats themselves. It is suggested, as a result of these studies that the insufficient fertility of saw-grass peats as compared with sedimentary peats should be looked for in some other factor rather than in the nature and abundance of the nitrogenous complexes in the two types of peat or in the rapidity of their relative decomposition and liberation of the nitrogen in an available form.

Allison and his associates (1) have shown that the addition of copper to saw-grass peat greatly stimulates the growth of cultivated crops; however, copper has no effect upon the microbiological activities in the peat itself. This shows further that we are dealing here with another limiting factor in the growth of plants on these two types of peat.

Smith (9) actually succeeded in demonstrating the existence in certain

types of peat of a substance which is distinctly toxic to plant growth; this toxicity or injurious action can be overcome by the use of copper. It still remains to be shown of course whether this specific injurious substance exists only in the one type of peat and not in the other. At least one fact remains certain that if such a substance exists in the saw-grass peat and not in the sedimentary type peat, its action upon the growth of certain higher plants must be much more marked than upon the growth of certain microörganisms in the soil.

TABLE 5

Moisture content, reaction, ash, and nitrogen content of sedimentary peat profile

NUMBER OF SAMPLE	DEPTH FROM SURFACE OF PEAT	MOISTURE CONTENT OF FRESH MATERIAL	REACTION OF FRESH MATERIAL	NITROGEN CONTENT OF DRY MATERIAL
	cm.	*per cent*	*pH*	*per cent*
80	1–30	97.82	7.50	3.40
81	30–60	97.75	7.55	3.00
82	60–120	90.90	7.25	2.41
83	120–180	91.46	7.06	2.48
84	180–240	95.45	7.15	2.56
85	240–300	94.23	7.28	2.67

TABLE 6

Chemical composition of the various layers of the sedimentary peat profile

(Percentage calculated from total of dry material)

NUMBER	ETHER SOLUBLE FRACTION	ALCOHOL SOLUBLE	HOT WATER SOLUBLE	HEMICELLULOSE	LIGNIN-LIKE COMPLEXES	PROTEIN	ASH	SUM
80	0.12	0.15	1.09	13.96	27.02	21.25	18.81	82.40
81	0.16	0.37	0.94	12.87	32.50	18.75	24.44	90.03
82	0.41	0.61	0.71	4.17	35.19	15.06	39.56	95.71
83	0.59	1.02	0.60	2.40	52.58	15.50	23.30	95.99
84	0.84	0.96	0.59	4.01	63.22	16.00	8.79	94.41
85	0.62	1.55	0.76	3.34	51.55	16.69	14.73	89.24

The second peat profile from Florida, namely, the allochtonous or Gyttja peat from Mud Lake was also subjected to a chemical analysis, using the methods previously (10) outlined. The results are given in tables 5 and 6.

The whole profile was practically free from cellulose, with the exception of mere traces in the upper layers. The low content of ether, alcohol, and water soluble materials, the high content of ash, protein and lignin-like complexes characterize this pure sedimentary profile as well as they do the sedimentary layers of other profiles. It is interesting to compare these figures with the composition of the Gyttja layers (A_6) of the Newton peat profile (11), which had 0.36 per cent ether-soluble material, 1.24 per cent water-soluble material, no cellulose, 5.92 per cent hemicellulose and 9.81 per cent protein. The

difference in the ash content in the different sedimentary peats will explain, to a large extent at least, certain of the differences in composition.

This formation was found to represent a number of highly interesting chemical and microbiological phases and it is our hope to come back to it later, for more extensive studies.

SUMMARY

1. The results of chemical analyses of an Everglade peat profile and a pure sedimentary (Gyttja) lake profile are reported.

2. The Everglade peat profile contains small amounts of cellulose only in the upper layers of saw-grass peat but none in the plastic and lower fibrous layers.

3. The chemical composition of the saw-grass layers of the Everglade peat is similar to other lowmoor peats. It is characterized by a low content of ether-, alcohol-, and water-soluble constituents, a medium hemicellulose and ash content and a considerable protein and lignin content. The plastic or sedimentary layer, however, is characterized by a low hemicellulose content and an exceptionally high ash content. The content of lignin-like complexes and proteins, when calculated on an ash free basis, are similar to the saw-grass peat layers.

4. In the decomposition of both the saw-grass and sedimentary layers of the Everglade peat, there is a rapid evolution of nitrogen in an available form and its accumulation as nitrate. The ratio between the carbon liberated as carbon dioxide and the nitrogen changed to nitrate is very narrow (1.3:1 to 3.3:1), much more so than in the decomposition of organic matter in ordinary soil.

5. There is no marked difference in the rapidity of liberation of nitrogen in an available form in the saw-grass and sedimentary layers of the Everglade peat profile. Any difference in fertility of soils produced by drainage and cultivation of the saw-grass and sedimentary types of peat and any favorable effect of copper upon plant growth should be looked for not in any difference in the activities of microörganisms or liberation of nitrogen from the organic complexes of the peat, but as due to some other factor.

6. An analysis of the chemical composition of a sedimentary peat profile taken from a lake in Florida, outside of the Everglade region, shows a similarity of this peat to sedimentary (Gyttja) layers of other peats, namely freedom from cellulose, a low content of ether-, alcohol-, and water-soluble constituents, a fairly high hemicellulose content, a high ash, protein, and lignin content.

REFERENCES

(1) Allison, R. V., Bryan, O. C., and Hunter, J. H. 1927 The stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulphate and other chemicals. Bull. 190, Florida Agr. Exp. Sta.

(2) Baldwin, M., and Hawker, H. W. 1915 Soil survey of the Fort Lauderdale Area, Florida. Advance Sheet, Bureau of Soils.

(3) DACHNOWSKI, A. P. 1924 The chemical examination of various materials by means of food-stuff analysis. *Jour. Agr. Res.* 29: 69–83.

(4) FORSAITH, C. C. 1916, 1917 A report of some allochtonous peat deposits of Florida. *Bot. Gaz.* 62: 33–52; 63: 190–208.

(5) HAMMAR, H. E. 1929 Some chemical and physical properties of the Florida Everglades soils. To be published in this Journal.

(6) MILLER, C. F. 1918 Inorganic composition of a peat and of the plant from which it was formed. *Jour. Agr. Res.* 13: 605–609.

(7) ROSE, R. E. 1912 Analyses of Florida Everglades soils. Ann. Rpt. State Chemist. of Florida, p. 140–148.

(8) SIEVERS, S. J., AND HOLTZ, H. F. 1926 The significance of nitrogen in soil organic matter relationships. Bul. 206, Washington Agr. Exp. Sta.

(9) SMITH, W. S. 1927 Een onderzoek naar het voorkomen en de oorsaken van de verschijnselen, welke worden aangeduid met den naam "Ontginningsziekte," Wageningen.

(10) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: I. Chemical nature of organic complexes in peat and methods of analysis. *Soil Sci.* 26: 113–137.

(11) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: II. Chemical composition of various peat profiles. *Soil Sci.* 26: 239–251.

MICROBIOLOGICAL ACTIVITIES IN THE SOIL OF AN UPLAND BOG IN EASTERN NORTH CAROLINA[1]

IVAN V. SHUNK

North Carolina State College

Received for publication January 9, 1929

In the autumn of 1924, Dr. B. W. Wells and the writer began an ecological study (22) of an upland grass-sedge bog located near Burgaw, Pender County, North Carolina. The vegetation on this area was decidedly unusual as compared with the type of vegetation found on most of the other parts of the coastal plain region of North Carolina, and pointed to an interesting complex of causal factors.

In connection with this ecological study the writer began the study of the microbiological activities of the soil of this upland bog. These soil studies were continued until the autumn of 1927 and form the basis of this paper.

DESCRIPTION OF THE BOG AREA

Location. The bog, known locally as the "Big Savannah," is located about 2 miles north of Burgaw, Pender County, North Carolina. It is on a part of the Wicomico terrace of the coastal plain region. This terrace was raised from the ocean during the Pleistocene Age. The bog is located a few miles west of the eastern edge of this terrace, and is now 24 miles from the ocean.

Size and topography. The area covered by the main bog is about 2½ miles long by 1 mile in width, comprising about 1,600 acres. The general shape of the area is in the form of a capital Y.

The surface of the bog is 17.7 meters above sea level. Although the main part of the bog is almost entirely level, the surface is roughened by innumerable small tussocks and ridges which are rarely over 10 cm. high. Scattered over the area there are numerous ant-hills which are 15 to 20 cm. high.

The bog is one of the highest areas in Pender County. On every side of the bog we find the beginnings of small streams which have their origin in shallow swamps, only a few centimeters to a meter or two below the surface of the main part of the bog.

Vegetation. The bog has been called a savannah by local people, and more commonly the "Big Savannah." It is not, however, a true savannah, as a savannah is a grassland with trees on it, whereas the bog in question is

[1] Part of a thesis submitted to the faculty of Rutgers University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

almost free from trees. Its general aspect is rather that of a prairie. The plants on the bog are representatives of about one hundred species, mostly perennials. The dominant plants are grasses and sedges. Species of *Carex* are however entirely absent, and leguminous plants never occur on the bog proper. A complete list of the species of plants found on this area is given by Wells and Shunk (22).

The grass-sedge complex is kept on the bog due to frequent fires which occur almost once a year and keep the bog practically treeless.

Soil. The soil of the bog is of the type known as "Portsmouth very fine sandy loam." It has been formed from the very fine sand laid down in deep water 15 to 20 miles off shore, before the Wicomico terrace was raised above the level of the ocean. A partial mechanical analysis of the surface soil made by Wells and Shunk (22) gave the following:

	MILLIMETERS	PER CENT
Fine gravel	2 –1	0
Coarse sand	1 –0.5	0
Medium sand	0.5 –0.25	5.4
Fine sand	0.25–0.1	24.5
Very fine sand, silt, clay, etc	0.1 and less	70.1

The subsoil is a mottled gray and yellow very fine sandy clay to a depth of a meter and more.

The carbon content of the surface soil as found by analysis is 3.26 per cent of the dry weight of the soil. The total nitrogen content is 0.24 per cent, thus giving a carbon-nitrogen ratio of 13.6 : 1 as compared to the usual average 10 : 1 ratio of cultivated fertile soils.

One of the most marked characters of the soil of this upland bog is the thick dark humus layer. This layer is from 35 to 40 cm. thick in the middle of the bog. The greater part of the roots of the grasses and other plants is found in this dark soil layer, and it is doubtless due to the partial decomposition of these roots that this thick layer containing humus has been accumulated. In most other types of areas of the coastal plain region the humus layer is rarely over 10 cm. thick.

On account of the very level surface of the whole bog, and the practically impervious subsoil, the area has very poor drainage. Much of the rain that falls on the bog remains there until lost by evaporation, or by the transpiration of plants. For this reason anerobic conditions prevail in this soil for a considerable part of the time, each heavy rain nearly or completely filling the bog with water. Even in dry weather, on account of the fineness of the soil particles causing good capillary action, the upper part of the bog remains moist, except in such very unusual seasons as the summer of 1926, when the water table fell to a meter and more below the surface. During this time the surface became quite dry.

HISTORICAL

So far as the writer has been able to learn, there has been little work done previously on the microbiology of the soils of upland bogs such as the soil of the "Big Savannah," and other grass-sedge bogs of eastern North Carolina. There have been made in North Carolina, however, some studies giving an idea of the extent of ammonification of nitrification in soils similar to the soil with which this paper deals.

Stevens and Withers (13) in 1909 in a series of tests of ammonification and nitrification, collected among other samples, one from a "savannah" in Craven County, N. C. This sample was tested for its ability to cause nitrification when inoculated into a suitable sterilized soil to which ammonium sulfate had been added. A further test was made to determine its ability to nitrify ammonium sulfate added to the soil itself, and incubated at optimum moisture concentration for four weeks. The results of the tests were as follows: (*a*) No nitrification occurred in the soil containing ammonium sulfate after inoculation with an infusion from the "savannah" soil. (*b*) When ammonium sulfate was added to the soil itself, no nitrate was found after four weeks of incubation.

Willis (24) has studied the relations existing between nitrification and acidity in certain muck lands of eastern North Carolina. In cut-over swamp land that has been drained, Willis found nitrate nitrogen in soil with an acidity as great as pH 3.62. He found that a sort of equilibrium existed about pH 4.0. If a small amount of lime was added, and the reaction made less acid than pH 4.0, nitrification was accelerated and the production of nitric acid from the large supply of organic nitrogen on hand again brought the pH to approximately 4.0.

EXPERIMENTAL WORK

The experimental work was carried out by studies of the following:

I. The numbers of bacteria, actinomyces and fungi in the soil under different conditions of treatment.
II. Effect of different soil treatments on the formation of ammonia and nitrates.
III. Rate of activities of soil microörganisms under different treatments as evidenced by the amount of carbon dioxide evolved.
IV. Character and amount of the soil organic matter.
V. Decomposition of cellulose added to the soil.
VI. Nitrogen fixation.

I. Numbers of microörganisms

Eight samples of soil were taken to a depth of about 15 cm., sifted through a 2 to 3 mm. sieve and composited. The moisture content of this soil when taken to the laboratory was 9.9 per cent on the basis of dry soil. By the use of Hilgard cups it was found that the water holding capacity of this soil was 63.1 per cent on the basis of dry soil.

The moisture content of the soil was brought to 60 per cent of its water holding capacity by the addition of distilled water. Twelve pots were then filled by using 2500 gm. of moist soil per pot. Each of the pots was then inoculated with a suspension of soil from a local greenhouse (State College, Raleigh, N. C.) mixed with soil from a cultivated field near the "savannah bog."

The soil in the pots was treated as follows:

Pot number	
1	Untreated
2	Untreated
3	Plus 0.5 per cent of calcium carbonate
4	Plus 0.5 per cent of calcium carbonate
5	Plus 0.10 per cent of sodium nitrate
6	Plus 0.10 per cent of sodium nitrate
7	Plus 0.5 per cent of calcium carbonate and 0.10 per cent of sodium nitrate
8	Plus 0.5 per cent of calcium carbonate and 0.10 per cent of sodium nitrate
9	Sub-optimum moisture, 40 per cent of saturation
10	Sub-optimum moisture, 40 per cent of saturation
11	Flooded
12	Flooded

These pots were kept in the incubator room at a temperature of approximately 25°C. for a little over four months, and then kept at room temperatures for the remainder of the experiment.

Throughout the course of the experiment samples of soil from the different pots were plated at eight different intervals and the numbers of bacteria, actinomyces and fungi were determined. The media used were acid agar [Waksman (15)] for the determination of the fungi, and albumen agar [Waksman and Fred (16)] for the determination of the bacteria and actinomyces.

Table 1 gives a summary of the averages of the numbers of microörganisms found in the eight different platings, and also the pH of the soils at the close of the experiment. The reaction of the soils was determined by the use of the quinhydrone electrode. Table 2 is given to show the fluctuation in numbers of the bacteria in the case of soil with optimum moisture, with lime added and optimum moisture, and under flooded conditions.

The addition of calcium carbonate caused a great increase in the numbers of bacteria. As shown by figure 1 in which the numbers of the bacteria in the soil that had been limed and in the untreated soil are compared, at the end of the first 26 days the bacterial numbers are about one hundred times as high for the limed soil as for the unlimed. From that time until the 181st day, there is a fairly large uniform decrease in the numbers in the limed, as compared with a slight decrease for the unlimed soil. From the 181st day to the 291st day, the numbers show a steady rise for the limed soil, whereas the numbers in the untreated soil show merely a slight increase.

It is evident that many of the organisms present in the soil at the beginning, or added in the inoculum, found conditions favorable in the limed soil, and

thus gave the tremendous initial increase in the numbers. However, after a few weeks they decreased quite rapidly, due perhaps to the using up of certain of the more readily available food materials. This decrease seems

TABLE 1

Summary of averages of numbers of microörganisms present per gram of dry soil in the pots during the period October 29, 1926 to August 16, 1927

TREATMENT	POT	BACTERIA AND ACTINOMYCES	ACTINOMYCES	FUNGI	pH AT CLOSE OF EXPERIMENT
None	1	2,558,000	327,000	81,000	5.2
	2	2,565,000	388,000	87,000	5.25
Plus lime	3	109,200,000	474,000	74,000	7.4
	4	126,900,000	512,000	77,000	7.3
Plus nitrate	5	7,860,000	513,000	138,000	4.7
	6	2,208,000	431,000	107,000	4.95
Plus lime and nitrate	7	104,900,000	392,000	92,000	7.3
	8	118,070,000	449,000	94,000	7.4
Sub-optimum moisture	9	1,588,000	389,000	80,000	4.8
	10	1,482,000	284,000	93,000	4.85
Flooded	11	1,243,000	410,000	57,000	5.6
	12	1,029,000	425,000	55,000	5.75

TABLE 2

Fluctuation in numbers of bacteria per 1 gram of dry soil in drained, drained and limed, and in flooded soil

INCUBATION	DRAINED (AVERAGE OF POTS 1 AND 2)	DRAINED AND LIMED (AVERAGE OF POTS 3 AND 4)	FLOODED (AVERAGE OF POTS 11 AND 12)
days			
26	1,935,000	233,250,000	1,050,000
61	1,963,000	193,624,000	533,000
88	1,450,000	143,650,000	680,000
116	1,545,000	136,725,000	415,000
150	760,000	49,650,000	652,000
181	2,796,000	22,600,000	1,012,000
239	2,825,000	60,330,000	910,000
291	4,320,000	101,833,000	995,000
Averages for period	2,199,000	117,708,000	781,000

not to have been due to a decrease in the amount of mobilized nitrogen, for the amount of ammoniacal and nitrate nitrogen was actually greater during the period of bacterial depression than earlier in the experiment when the numbers of bacteria were higher.

The lowest part of the curve in figure 1 for the limed soil is correlated with the temperature conditions under which the soil was kept. On March 12, 1927, on the 134th day of the experiment, the pots were removed from the incubator room where they had been kept at a temperature of approximately 25°C. The soils were then transferred to glass jars and transported to New

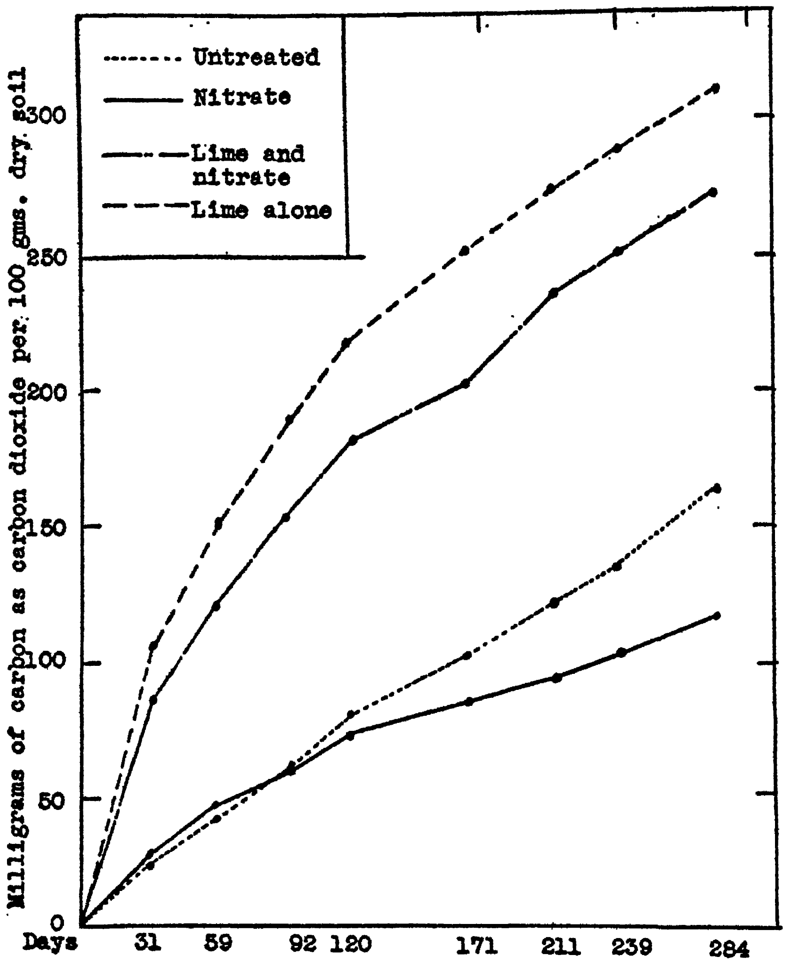


FIG. 1. NUMBERS OF BACTERIA IN UNLIMED AND LIMED SOILS BASED ON THE AVERAGES OF POTS 1 AND 2, AND OF POTS 3 AND 4

Jersey in an open car. On their arrival at the New Jersey Experiment Station they were transferred to pots and kept in the laboratory at room temperatures for the remainder of the experiment. During the trip from Raleigh, N. C. to New Brunswick, N. J. the temperature dropped to around the freezing point, or below, and the room temperature at New Brunswick was nearer

20° than 25°C. until well into the summer. Later in June, July, and August the general increase in the summer temperature brought the room temperature to a higher figure, the rise in temperature at this time being correlated with the rise in bacterial numbers.

In a second pot experiment soil was treated with 0.10 per cent of ammonium sulfate and 1 per cent of calcium carbonate and kept in a pot for approximately 18 months. It was kept at optimum moisture concentration for three months and then allowed to become quite dry (about 20 per cent of saturation).

Table 3 gives the numbers of microörganisms present as shown by plating this soil at the beginning and end of the 18-month period.

After 18 months of incubation, whitish spots were noticed on the rather dry lumps of soil. If a little of this whitish material was scraped from a lump of soil, moistened with a drop of water, and examined microscopically, either unstained or stained by the addition of a drop of methylene blue, it was found that there was an abundance of actinomyces growth. Some of the whitish material was scraped off, fixed to the slide with a dilute agar solution, and then stained with erythrosin. Large masses of actinomyces were seen. The masses became stained in such a way as to look very similar to large groups of spherical or short rod-shaped bacteria. In fact, if one had not seen the unstained preparations, he might readily have considered these masses of actinomyces as bacteria, and counted them as such.

As shown by table 3, the plate method using albumen agar gave only 300,000 actinomyces per gram in this soil, although microscopic examination showed them to be very numerous. Two suggestive conclusions may be drawn from the examination of this sample of soil: (*a*) When stained preparations of soils are examined, the masses of so-called short rod-shaped or spherical bacteria may be in reality masses of actinomyces; and (*b*) large numbers of actinomyces sometimes fail to grow on the albumen agar used for making plate counts.

According to the results of the microscopic examination, although it was not found possible to determine even approximate numbers, the number of actinomyces must have increased greatly in this soil under its treatment. The soil sample at the end of the period had a pH of 7.53, determined by the quinhydrone method. The slightly alkaline reaction together with the dry condition of the soil with its accompanying improved aeration, was thus shown to favor the abundant development of actinomyces.

II. Ammonia and nitrates

Samples were taken at intervals from the series of pots in which the fluctuation of the numbers of microörganisms was studied, and determinations were made of the ammoniacal and of the nitrate nitrogen present. The amount of ammonia was determined by leaching the soil with half-normal potassium chloride solution, adding magnesium oxide and distilling the ammonia into standard acid. Nitrates were determined by the usual phenoldisulphonic acid method.

The fluctuations in the amounts of ammoniacal and nitrate nitrogen from time to time are given in table 4. It is interesting to note that no nitrification occurred in the soil of any of the pots except those to which lime had been

TABLE 3

Microörganisms in soil receiving ammonium sulphate and lime

Figures based on 1 gram of dry soil

INCUBATION PERIOD	BACTERIA AND ACTINOMYCES	ACTINOMYCES	FUNGI
None	1,103,000	20,000	13,400
18 months	25,900,000	300,000	40,000

TABLE 4

Milligrams of N as ammonia and as nitrate per 100 grams dry soil

INCUBATION	UNTREATED (AVERAGE OF POTS 1 AND 2)		LIMED (AVERAGE OF POTS 3 AND 4)		NITRATE ADDED (POTS 5 AND 6)		LIMED AND NITRATED ADDED (POTS 7 AND 8)		SUB-OPTIMUM MOISTURE (POTS 9 AND 10)		FLOODED (POTS 11 AND 12)	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
days												
62	0.8	None	8.2	None	5.5	15.7	7.2	15.2	0.7	None	0.8	None
118	1.8	None	3.2	5.8	7.3	20.2	10.3	21.1	1.1	None	1.5	None
181	2.3	None	0.3	12.1	8.8	14.9	1.3	23.7	1.5	None	2.7	None
237	2.9	None	0.4	11.2	9.5	12.1	0.2	22.2	1.6	None	2.9	None
293	4.7	None	0.7	13.3	11.2	11.7	0.6	24.7	2.2	None	4.3	None

TABLE 5

Milligrams of carbon evolved as carbon dioxide per 100 grams of dry soil in a period of 284 days

FLASK NUMBER	TREATMENT	C AS CO_2 EVOLVED
1	Untreated	164.0
2	Untreated	162.3
3	Limed	225.4
4	Limed	234.7
5	Sodium nitrate added	114.6
6	Sodium nitrate added	120.8
7	Limed and nitrate added	194.8
8	Limed and nitrate added	201.4
9	Sub-optimum moisture	111.8
10	Sub-optimum moisture	121.9
11	Flooded	107.8
12	Flooded	110.9

added. In pots 3 and 4, which had been limed, but had been given no addition of nitrates, the soil showed an accumulation of 14 mgm. of nitrogen as nitrate per 100 gm. of dry soil. Since 22.7 mgm. of nitrogen were added to pots 7 and 8 per 100 gm. of soil, one would have thus expected to find some-

thing like 36.7 mgm. of nitrate nitrogen per 100 gm. of soil, unless some of it had been reduced or lost. On the other hand, the average amount of mobilized nitrogen in the soil of pots 7 and 8 at the close of the experiment was 25.3 mgm. per 100 gm. of soil. Undoubtedly, a considerable amount of

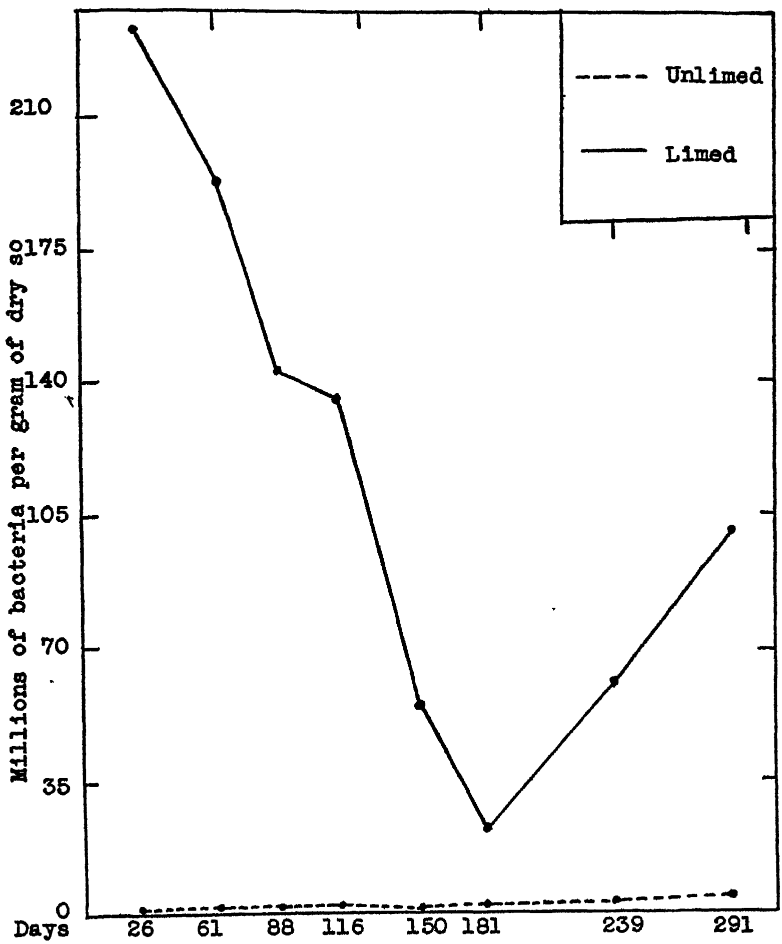


FIG. 2. EFFECT OF LIMING AND ADDITION OF NITRATES ON THE EVOLUTION OF CARBON DIOXIDE

the ammonia, nitrate nitrogen, or of both must have been immobilized by being built up into microbial protoplasm.

III. Carbon dioxide evolution

From each of the 12 pots used for determination of numbers of microörganisms, and for the study of ammonia and nitrate formation, enough moist

soil to be equivalent to 100 gm. of dry soil was taken and placed in a flask. These flasks were connected with aeration apparatus and the evolution of carbon dioxide determined from week to week by the method of Waksman and Starkey (19).

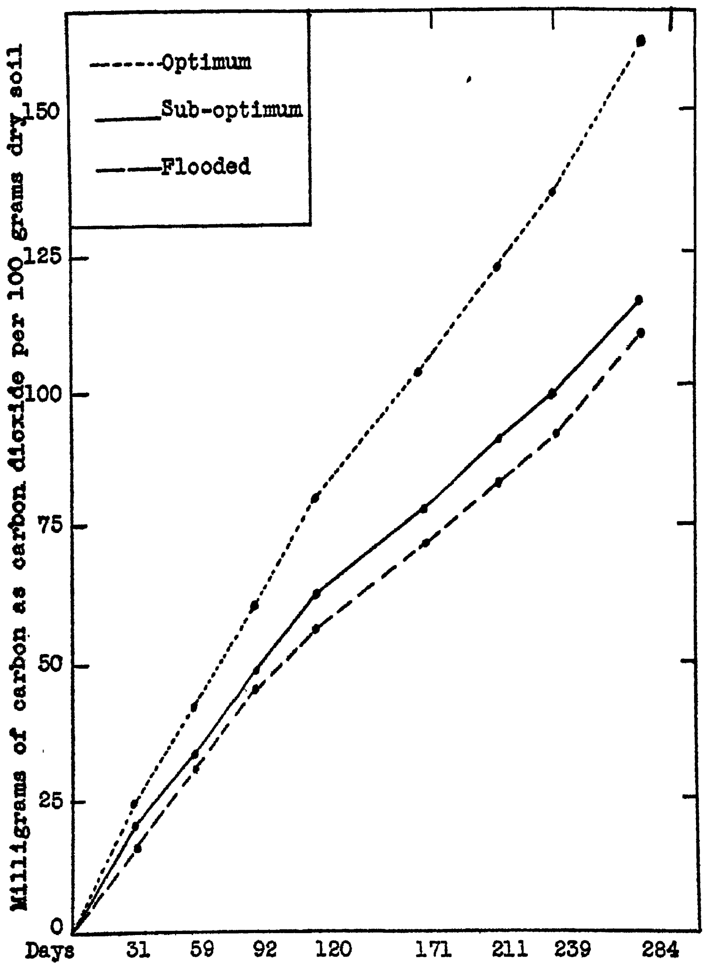


FIG. 3. EFFECT OF AMOUNT OF MOISTURE ON EVOLUTION OF CARBON DIOXIDE

Table 5 gives the total carbon evolved as carbon dioxide per 100 gm. of soil in each of these flasks in a total period of 284 days. In those soils to which lime had been added, the amount of residual CO_2 in the lime was determined and the amount liberated due to interaction with soil acids was deducted from the totals. The figures for carbon dioxide evolved represent only that part which is attributable to the activity of microörganisms.

Figure 2 shows the effect of liming and addition of nitrate on the evolution of carbon dioxide, and figure 3 the effect of amount of moisture in the soil on the amount of CO_2 evolved.

Liming the bog soil caused a considerable increase in the loss of carbon as carbon dioxide. This increase of evolved carbon dioxide was correlated with a large increase in the numbers of bacteria and also of actinomyces present.

The addition of sodium nitrate to furnish available nitrogen for microbial use was shown to have a depressing effect on the rapidity of carbon dioxide production. As shown by figure 2, for nearly three months the amount of carbon dioxide was slightly in excess of that evolved from the untreated soil, but after about eighty days the amount of carbon dioxide dropped considerably below the amount from the untreated sample. When lime was added in addition to the nitrate, there was also a depressing effect so far as evolution of carbon dioxide was concerned.

The depressing effect on carbon dioxide evolution is probably due to a slight toxicity resulting from the amount of nitrate used.

IV. Soil organic matter studies

Following the methods worked out by Waksman (20), studies were made of the soil organic matter of the soil of the upland grass-sedge bog. The soil samples were extracted with hot alkali, both after and before treatment with hydrochloric acid. Extractions were made as follows: 50 gm. of dry soil were treated with 100 cc. of 1 per cent HCl solution and the acid allowed to act over night. The supernatant liquid was then decanted on a filter, and the soil washed with an additional 100 cc. of the HCl solution and then with about 20 cc. of water, and transferred to the filter. The soil was removed from the filter and dried in the oven. To each of the samples of dry soil, either with or without the previous acid treatment, were added 100 cc. of 2 per cent NaOH solution, and extraction carried out in the autoclave at 15 pounds pressure for 30 minutes. The supernatant liquid was decanted on a filter, and the residue, after washing with water, was then added to the filter. A second extraction was made by using 100 cc. of 4 per cent NaOH in the autoclave for 30 minutes. The extract was then decanted on the same filter and the soil washed with 50 cc. of water and transferred to the filter. The filter was washed with hot 1 per cent NaOH, and then with hot water. The filtrate was treated with an excess of 1–1 HCl and boiled for 20 minutes after which the precipitate, the so-called α-fraction, was filtered on a weighed paper, dried, and weighed. The filtrate after the removal of the α-fraction was treated with sufficient NaOH solution to bring the reaction to approximately the neutral point, and the precipitate, the β-fraction, filtered off, dried and weighed.

The total nitrogen in the α- and β-fractions was determined by the Kjeldahl method, and the total carbon by the method of Waksman and Starkey (18).

Table 6 gives the amount of the various fractions and their nitrogen and carbon content.

The preliminary treatment with 1 per cent HCl solution for a period of nearly twenty-four hours had little effect on the amount of α-fraction extracted by the alkali. This was in accord with the findings of Waksman (20) who reports even a slightly diminished yield of α-fraction following treatment with hydrochloric acid. The acid treatment did, however, increase the amount of the β-fraction that was extracted by the alkali.

Of the total organic matter in the soil the α-fraction yielded almost 20 per cent. The percentage of nitrogen in the α-fraction was found to be close to 3 per cent. This percentage of nitrogen in the α-fraction agrees closely with that found by others, Waksman (20) and Joseph and Whitfeild (11).

The carbon content of the α-fraction was found to be almost exactly 60 per cent. In comparison with this percentage of carbon, Joseph and Whitfeild (11) found approximately 57 per cent carbon in the "humus" extracted from three different soils in Africa.

TABLE 6

Soil organic matter extracted by alkali solution from 50 grams of dry bog soil under 15 pounds pressure, both with and without previous treatment with hydrochloric acid

SAMPLE	HCl TREATMENT	α FRACTION			β FRACTION		
		Yield	Nitrogen	Carbon	Yield	Nitrogen	Carbon
		grams	*per cent*	*per cent*	*grams*	*per cent*	*per cent*
1	Plus	0.790	3.1	....	0.964	0.1	....
2	Plus	0.820	3.0	....	0.829	0.1	....
3	Plus	0.773	...	61.0	1.166	...	1.95
4	Plus	0.809	....	59.4	0.827	...	2.0
5	Minus	0.769	2.9	....	0.725	0.1	....
6	Minus	0.720	3.0	....	0.528	0.4	....
7	Minus	0.743	...	59.0	0.805	...	3.55
8	Minus	0.764	...	60.7	0.559	...	2.25

The ash content of the α-fraction was found to be low, 1.4 to 2.2 per cent, and mght have been lowered by repeated washing of the fraction with HCl. A low ash content was to be expected from the work of Waksman (20) and of Joseph and Whitfeild (11). These authors obtained an ash content of less than 1 per cent.

In ordinary fertile soils, the *carbon-nitrogen* ratio usually varies between 8:1 and 12:1 as determined by various analyses made by a number of investigators. This ratio is usually given as approximately 10:1.

The total carbon in the soil of the "savannah" bog was found to be 3.26 per cent, and the nitrogen 0.24 per cent, thus giving a carbon-nitrogen ratio of 13.6 : 1. This wide ratio is largely the result of the more or less continued anaerobic conditions in the soil of the bog. Under these conditions a large

part of the carbon-complexes are broken down to intermediate products instead of carbon dioxide, and the carbon is thus left in the soil. The lignins especially tend to accumulate, as they are attacked only very slowly by anaerobic microörganisms. Since the organic matter in the soil has this wide ratio of carbon to nitrogen, when more organic matter in the form of roots and leaves is added, the decomposition is slow because of the lack of nitrogen available for the use of the microörganisms. On account of the wide carbon-nitrogen ratio in the plant debris added to this soil, and the slow decomposition of this organic matter, the tendency is to widen rather than narrow the carbon-nitrogen ratio of this soil.

V. Decomposition of cellulose added to the bog soil

Two methods of approach were used in studying the decomposition of cellulose added to the soil in the form of filter paper: (*a*) by determining the

TABLE 7

Carbon as CO_2 evolved; microörganisms present, and mobilized N at close of experiment

TREATMENT	MGM. OF C AS CO_2 EVOLVED PER 100 GRAMS DRY SOIL	BACTERIA PER GRAM DRY SOIL	FUNGI PER GRAM DRY SOIL	MGM. N AS NITRATE PER 100 GM. DRY SOIL	MGM. N AS AMMONIA PER 100 GM. DRY SOIL
Filter paper only	27.1	1,047,000	61,000	None	0.4
	28.9	749,000	76,000	None	0.4
Filter paper and 21.7 mgm. N as NO_3 added	55.3	579,000	1,500,000	17.2	1.7
	66.3	315,000	1,828,000	18.7	1.9

rate of carbon dioxide production, and (*b*) by determining the residual cellulose after a period of incubation.

1. Carbon dioxide production by soil containing filter paper. Samples of moist soil weighing 100 gm., with a moisture content of approximatey 50 per cent of saturation, were placed in each of four flasks.

Nos. 1 and 2. One gram of filter paper added.
Nos. 3 and 4. One gram of filter paper and 100 mgm. of sodium nitrate added.

The flasks were filled and allowed to stand in the laboratory. At intervals during a period of 33 days determinations were made of the CO_2 evolved. At the close of the experiment determinations were made of the numbers of microörganisms present, and of the mobilized nitrogen. The results of these determinations are given in table 7.

The addition of nitrogen in the form of nitrate caused a speeding up of the decomposition of the cellulose as evidenced by the increase in evolution of carbon dioxide. This increase in rate of CO_2 production may be due to the increase in the fungous activity as indicated by the increase in the numbers

of fungi found, but may be due also to the activity of cellulose decomposing bacteria, which would not grow on the media used for making the microbial counts.

This experiment indicates that the rate of decomposition of cellulose in this soil is dependent on available nitrogen rather than on the types of organisms present.

2. *Residual cellulose after incubation.* Four pots were filled with 1,200 gm. of moist soil on September 18, 1926. The soil was from the same composite sample from which the pots in the pot experiment were filled. To each of the pots enough filter paper was added to be equivalent to 1.29 gm. of pure cellulose per 100 gm. of dry soil.

Pots 13 and 14. Soil contained a slight excess of water for 15 days; afterwards, optimum moisture.

Pots 15 and 16. Soil kept flooded throughout the course of the experiment.

TABLE 8

Residual cellulose in 100 grams of dry soil

POT	TREATMENT	GRAMS OF CELLULOSE AFTER 204 DAYS	GRAMS OF CELLULOSE AFTER 310 DAYS
1	No cellulose added. Optimum moisture	0.0144	......
2	No cellulose added. Optimum moisture	0.0192	......
13	1.29 grams of cellulose added. Optimum moisture	......	0.7610
14	1.29 grams of cellulose added. Optimum moisture	0.8248	0.7807
15	1.29 grams of cellulose added. Soil flooded	0.9780	0.8651
16	1.29 grams of cellulose added. Soil flooded	0.7920	0.6960

About seven months, 204 days, after the filter paper was added, enough moist soil was taken from each of the pots to be equivalent to 50 gm. of dry soil. Samples were taken also from pots 1 and 2 of the pot experiment for determining numbers of organisms etc., to be used as controls. Pots 1 and 2, containing the same soil as the pots with added cellulose, were kept at optimum moisture concentration and treated similarly to pots 13 and 14. The only essential difference was that pots 1 and 2 had received no filter paper.

The amount of cellulose present in these samples of soil was determined by the method given by Waksman and Heukelekian (17).

Three hundred and ten days after the experiment was begun, another set of determinations of residual cellulose were made on the soil from pots 13, 14, 15, and 16. Table 8 gives the amount of cellulose found in the different samples after 204 days and after 310 days. Slightly more of the cellulose was decomposed under aerobic than under anaerobic conditions.

Determinations of the ammoniacal and nitrate nitrogen present in the soils

in the pots showed very small amounts of ammoniacal nitrogen and no nitrates at all.

It is quite evident from the small amount of mobilized nitrogen in these samples, that it is the lack of available nitrogen, rather than the lack of cellulose decomposing organisms that accounts for the slow decomposition of cellulose in this soil. This conclusion is supported by the experiment on the rate of carbon dioxide production by soil with added cellulose both with and without the addition of sodium nitrate. In that experiment it was found that when nitrate was added, although the soil was not inoculated with any organisms, there was a marked increase in the rate of carbon dioxide production which indicated a speeding up of cellulose decomposition.

VI. Nitrogen fixation

There are no legume plants growing on the "savannah" bog; hence, any fixation of atmospheric nitrogen must be due to non-symbiotic microörganisms. The fact that the upper layers of the soil contain as much as 0.24 per cent of nitrogen, and none is ever added by man, indicates that atmospheric nitrogen must furnish a large part of this. Since there are two groups of bacteria, aside from nodule bacteria, that normally fix nitrogen, the aerobic *Azotobacter* type, and the anaerobic *Amylobacter* type, search has been made for both types of organisms.

1. Aerobic nitrogen fixing bacteria. Silica gel plates were prepared in accordance with the method used by Waksman and Carey (21). After the gels had been thoroughly washed in running water and in boiled distilled water, they were dried very slightly in the oven at 60°C. As soon as the surface of the gel was nearly dry, each plate was impregnated with 1 cc. of the following nutrient solution:

Dibasic potassium phosphate	1 gm.
Magnesium sulfate	0.2
Sodium chloride	0.2
Calcium chloride	0.1
Ferric chloride (5 per cent)	1 drop
Mannite	15 gm.
Distilled water	100

After the addition of the 1 cc. of the above nutrient solution to each of a number of plates of the gel, the plates were returned to the oven and kept there until the surface had become fairly dry. A little calcium carbonate was powdered over the surface, and the plates were then ready for inoculation.

The silica gels were inoculated with small bits of the upland bog soil and some of the plates with a pure culture of *Azotobacter*. The plates were then incubated in a moist chamber for almost four weeks.

Azotobacter colonies developed readily on the plates inoculated with the

pure culture, but failed to develop on any of the plates inoculated with the bog soil, thus indicating the absence of *Azotobacter*.

Several months later another batch of silica gel plates were inoculated with a fresh sample of the bog soil and gave the same negative results, even after 52 days of incubation. The reaction of the bog soil has been found to be close to pH 5.2, as determined by several tests with the quinhydrone electrode. According to the findings of others, Gainey (8, 9, 10) and Christensen (3, 4) *Azotobacter* would not be expected to be present in this soil on account of its reaction.

2. *Anaerobic nitrogen fixing bacteria.* Two series of experiments were carried out in regard to anaerobic nitrogen fixation, the first, to show the presence, or absence of organisms that could fix nitrogen under anaerobic conditions, and the second to try to find *B. amylobacter* and isolate it.

Series 1. A medium was prepared according to the following formula:

Distilled water	1000 cc.
Dextrose	20 gm.
Dibasic potassium phosphate	1
Magnesium sulphate	3
Sodium chloride	0.01
Manganous sulfate	0.01
Ferric chloride solution (10 per cent)	3–4 drops
Calcium carbonate	10 gm.

To each of 6 tumblers 100 cc. of the above medium was added, and sterilized in the autoclave. Each of the solutions in the tumblers was inoculated with a 5 cc. aliquot of a 5-minute soil suspension (100 gm. of soil added to 200 cc. of sterile distilled water, shaken for five minutes, and allowed to settle slightly). Four of the tumblers were placed in a large dessicator jar, and the oxygen absorbed by the addition of pyrogallic acid and sodium hydroxide. Determinations of total nitrogen were made on the solutions and inoculum of two of the tumblers, and gave a nitrogen content of 0.3 mgm. per tumbler. After 28 days of incubation under anaerobic conditions, the solutions in the other four tumblers were analyzed for nitrogen content and gave the following results: 3.85, 4.58, 4.35, and 3.45 mgm., respectively.

Since the inoculated solution at the beginning of the experiment contained 0.3 mgm. of nitrogen, there was a fixation of an average of 3.75 mgm. of nitrogen per 100 cc. of the solution containing 2 gm. of dextrose sugar.

Another experiment, conducted in the same way as the above, showed a fixation of approximately 4.2 mgm. of nitrogen per 100 cc. of solution. These two experiments prove quite conclusively that organisms which can fix atmospheric nitrogen under anaerobic conditions, are present.

Series 2. The method used to attempt the isolation of *B. amylobacter* was essentially that of Burri (2). A number of tubes were filled about one-half full of a medium having the following composition.

Dibasic potassium phosphate	3 gm.
Magnesium sulfate	0.2
Sodium chloride	0.01
Ferrous sulfate	0.01
Manganous sulfate	0.01
Dextrose	10
Calcium chloride	0.01
Agar-agar, Bacto	15
Distilled water	1000

After this medium was melted and allowed to cool partially, dilutions of the bog soil were mixed with it by shaking, and the agar allowed to set. A plug of poisoned agar of the following composition was then added.

Distilled water	100 cc.
Mercuric chloride	0.02 gm.
Agar-agar, Bacto	1.0 gm.

After incubation several of the shake-agar tubes showed colonies which gave rise to gas formation. By running a hot wire down along the side of the agar in one of these tubes, and then by shaking, the cylinder of agar was removed almost intact, and placed in a sterile dish. By the use of a flamed knife, a colony which had just begun to show gas formation was cut out of the cylinder of agar and transferred to a tube of nitrogen-free nutrient solution. By connecting this tube to another tube filled with raw potato, anaerobic conditions were soon established in both tubes. After a few days of incubation, a microscopic examination made by the nigrosin method, revealed a number of typical *B. amylobacter* organisms. The culture was, however, not pure.

Silica gel plates were prepared in the same way as for the *Azotobacter* tests except that dextrose sugar was used instead of mannite. In two of the plates inoculated with bits of the "savannah" soil, *B. amylobacter* was found to develop colonies next to the bottom of the plate.

GENERAL DISCUSSION

In the study of the upland grass-sedge bog, one of the points of interest has been to discover, if possible, how the upland bog conditions are essentially different from those of a lowland bog. In an upland bog such as the one studied, although each rain may fill the soil more or less completely with water, yet standing water does not remain on the soil continuously. We have a period of several days during which the soil is practically, or entirely saturated, followed by a few days in which the upper layers of the soil become partially dry and there is more or less aeration. On the other hand in a lowland bog the soil would be saturated more continuously, at least for longer periods at one time.

The accumulation of so much organic matter in the soil, as compared with the amount of organic matter in cultivated soils in the vicinity of the bog is due to the interaction of several factors. In the first place it is dependent on the type of vegetation which is composed of grasses, sedges and various other herbaceous plants. These plants continue to dominate the area, because fire sweeps over the bog almost every year and effectively prevents the trees which start to develop, from becoming established and shading out the grasses, sedges, etc. The masses of roots and underground stems of the herbaceous vegetation are found chiefly in the upper 30 to 40 cm. of the soil. Then, due in part to the more or less continuous anaerobic conditions, with their influence on microbial activities, the dead roots and other underground parts are only partially decomposed, thus allowing an accumulation of so-called humus, or soil organic matter. The accumulation of this organic matter then increases the water holding capacity of the soil, and tends to keep the soil too wet for good aeration, which would permit the rapid activity of aerobic microörganisms, thus aiding in the further accumulation of the humus.

The entire absence of leguminous plants from the grass-sedge bog is probably correlated with the inability of the legumes to withstand saturated soil conditions for several days at a time. The absence of all legumes should not be attributed to the effects of acidity, as there are certain species of this group of plants that can and do grow in more acid soils, and legume nodule bacteria which can do well at higher concentrations of hydrogen-ions [Fred and Davenport (7)]. In the absence of leguminous plants, the source of the nitrogen in the soil must be largely the fixation of atmospheric nitrogen by non-symbiotic nitrogen fixing microörganisms. No nitrogenous fertilizer is ever added to this soil, as it is never cultivated, and yet the nitrogen content of the upper layers of the soil is rather high (0.24 per cent of the dry weight of the soil). The burning over of the bog causes a loss of most of the nitrogen that is in the aerial parts of the plants. This loss constitutes the principal loss of nitrogen, since there are no nitrates present to be lost by denitrification, or by drainage.

From the experimental work it has been found that *Azotobacter* species are not present. This absence of *Azotobacter* is no doubt due to the acidity of this soil and the anaerobic conditions. Just as found by Gainey (8, 9, 10), Christensen (3, 4), and others *Azotobacter* will not remain in a soil with a pH more acid than 6.0, whereas the pH of this soil is approximately 5.2. On the other hand, anaerobic nitrogen fixing bacteria can develop and fix nitrogen under more acid soil conditions, as found by Dorner (6). The anaerobic nitrogen fixing organism, *B. amylobacter*, has been found in several different samples of the soil by the use of silica gel plates and by other methods. The activities of this organism furnish undoubtedly the most important source of nitrogen in this bog soil.

Nitrate nitrogen has not been found in this soil in nature, although a number of samples have been tested. These samples were taken from different

places in the bog and at different times of the year over a period of about two years. One might suspect that the absence of nitrates and of nitrifying bacteria might be due to the acidity of the soil. However, the work of Willis (24) on muck soils of North Carolina, as well as of other workers elsewhere, Stephenson (12), Abbott, Conner and Smalley (1), White (23), and Temple (14), shows that nitrification does occur in acid soils. While the acidity of the soil is probably one of the factors hindering nitrification, it is not the only factor. When lime is added to the soil in pots in sufficient amounts to neutralize the acidity, and the soil is kept at optimum moisture concentration, nitrification will become evident after an interval of 6 to 10 weeks. It is advantageous to inoculate the soil thus drained and limed with a suspension of fertile soil, because it has been shown experimentally that nitrifying bacteria may be entirely absent from this soil.

The carbon-nitrogen ratio of the soil is quite wide, almost 14:1 as compared with the 10:1 to 12:1 ratio of fertile soils. This wide carbon-nitrogen ratio is undoubtedly one of the principal factors in the checking of nitrification. Just as found by Clark and Adams (5), a wide carbon-nitrogen ratio tends to check nitrification. Under these conditions the microörganisms use up most of the available nitrogen and store it in their microbial protoplasm; therefore, little is liberated as ammonia, and without ammonia we cannot have nitrification. When lime is added, and the soil is kept at optimum moisture concentrations, conditions are made favorable for the activity of aerobic bacteria and of actinomyces, and since the bacteria can get along with less nitrogen per unit of carbon consumed as energy than the fungi [Waksman and Heukelekian (17)], more nitrogen will be liberated as ammonia. When the soil reaction is neutral and there is ammonia present, nitrification will take place.

When cellulose in the form of filter paper was added to the soil, its decomposition was very slow, both under aerobic and anaerobic conditions. By using the rate of evolution of carbon dioxide as an index of the rate of decomposition of the cellulose, this slow decomposition was found to be due to the lack of available nitrogen, since when nitrate nitrogen was added there was a considerable increase in the rate of carbon dioxide production, accompanied by a large increase in the number of fungi present.

The cellulose added to the soil in nature by the plant residues, mostly roots and other underground parts, since the tops are usually burned every year, is largely decomposed. Cellulose determinations made on the soil from the bog showed a low percentage of cellulose.

If the soil is limed and allowed to become quite dry, actinomyces become active. Since these organisms are probably the principal microörganisms that can attack the α-fraction of the soil organic matter, and they are hindered by lack of aeration and also by acidity, little decomposition of the α-fraction can occur in the bog soil under the conditions found in nature. This leads to an accumulation of the lignins and nitrogen complexes comprising the α-fraction.

The results of the experimental work indicate that if this upland bog could be properly drained and then limed, that the organic matter would be gradually decomposed with the liberation of nitrogen as ammonia. This ammonia would then be nitrified. However, since a considerable part of the nitrogen is tied up in the α-fraction of the soil organic matter, that part of the nitrogen would become available very slowly, and it would probably take several years of cultivation before a satisfactory yield of any ordinary crop plant could be obtained.

SUMMARY

A study has been made of the microbiological activities of the soil of an upland grass-sedge bog, and the modifications of these activities produced by the addition of lime and of nitrogen salts. The effects of drainage and flooding have been studied also.

Nitrification does not occur in this soil under natural conditions. It may be made to occur by liming and drainage as determined by pot experiments, but only after an interval of 6 to 10 weeks.

No leguminous plants are found growing on the bog and yet the soil contains 0.24 per cent of total nitrogen. The source of this nitrogen may be to some extent atmospheric nitrogen fixed by non-symbiotic organisms.

Azotobacter species are not present on account of the soil acidity and anaerobic conditions, the pH of the soil being about 5.2.

Bacillus amylobacter has been found in several samples of the soil.

The addition of enough lime to the soil to neutralize the acidity results in a great increase in the numbers of bacteria, but does not appreciably change the numbers of fungi.

Liming the soil markedly increases the rate of carbon dioxide production by the soil micröorganisms.

When the soil is saturated with water, or contains too little water, the rate of carbon dioxide production is reduced in comparison with soil at optimum moisture concentration.

The soil has a wide carbon-nitrogen ratio, 13.6 : 1, which tends to retard the decomposition of the soil organic matter.

When the soil is neutralized with lime and allowed to become quite dry, actinomyces become very numerous. These give the soil a whitish appearance.

Cellulose added to the soil in the form of filter paper is very slowly decomposed on account of the lack of available nitrogen.

ACKNOWLEDGMENTS

The writer desires to express his sincere appreciation of the valuable suggestions given by Dr. Selman A. Waksman, under whose direction this work was carried out. He is indebted also to Dr. B. W. Wells and Mr. L. G. Willis for aid in a part of the laboratory and field work.

REFERENCES

(1) Abbott, J. B., Conner, S. D., and Smalley, H. R. 1913 The reclamation of an unproductive soil of the Kankakee Marsh region; soil acidity, nitrification, and the toxicity of soluble salts of aluminum. Purdue Univ. Exp. Sta. Bul. 170.

(2) Burri, R. 1902 Zur Isolierung der Anäeroben. *Centbl. Bakt.* (II), 8: 533-537.

(3) Christensen, H. R. 1915 Studien über den Einfluss der Bodenbeschaffenheit auf das Bakterienleben und den Stoffumsatz im Erdboden. *Centbl. Bakt.* (II), 43: 1-166.

(4) Christensen, H. R. 1917 Experiments in methods for determining the reaction of the soil. *Soil Sci.* 4: 115-178.

(5) Clark, H. W., and Adams, G. O. 1912 The influence of carbon upon nitrification. *Jour. Ind. Eng. Chem.* 4: 272-274.

(6) Dorner, W. 1924 Beobacungen über das Verhalten der Sporen und vegetativen Formen von Bacillus amylobacter A. M. et Bredemann bei Nachweis-und Reinzuchtversuchen. *Landw. Jahrb. Schweiz.* 38: 1-28.

(7) Fred, E. B., and Davenport, A. 1918 Influence of reaction on nitrogen-assimilating bacteria. *Jour. Agr. Res.* 14: 317-336.

(8) Gainey, P. L. 1918 Soil reaction and the growth of *Azotobacter*. *Jour. Agr. Res.* 14: 265-271.

(9) Gainey, P. L. 1923 Influence of the absolute reaction of a soil upon its *Azotobacter* flora and nitrogen fixing ability. *Jour. Agr. Res.* 24: 907-937.

(10) Gainey, P. L. 1923 A study of the effect of changing the absolute reaction of soils upon their *Azotobacter* content. *Jour. Agr. Res.* 24: 289-296.

(11) Joseph, A. F., and Whitfeild, B. W. 1927 The organic matter in heavy alkaline soils. *Jour. Agr. Sci.* 17: 1-11.

(12) Stephenson, R. E. 1920 Nitrification in acid soils. Iowa Exp. Sta. Res. Bul. 58.

(13) Stevens, F. L., and Withers, W. A. 1912 Studies in soil bacteriology: V. The nitrifying and ammonifying powers of North Carolina soils. *Centbl. Bakt.* (II), 34: 187-203.

(14) Temple, J. C. 1914 Nitrification in acid or non-basic soils. Ga. Agr. Exp. Sta. Bul. 103.

(15) Waksman, S. A. 1922 A method for counting the number of fungi in the soil. *Jour. Bact.* 7: 339-341.

(16) Waksman, S. A., and Fred, E. B. 1922 A tentative outline of the plate method for determining the number of microörganisms in the soil. *Soil Sci.* 14: 27-28.

(17) Waksman, S. A., and Heukelekian, O. 1924 Microbiological analysis of soil as an index of soil fertility: VIII. Decomposition of cellulose. *Soil Sci.* 17: 275-291.

(18) Waksman, S. A., and Starkey, R. L. 1923 On the growth and respiration of sulfur-oxidizing bacteria. *Jour. Gen. Phys.* 5: 288-290.

(19) Waksman, S. A., and Starkey, R. L. 1924 Microbiological analysis of soil as an index of soil fertility: VII. Carbon dioxide evolution. *Soil Sci.* 17: 141-161.

(20) Waksman, S. A. 1926 On the origin and nature of soil organic matter or soil "humus": II. Method of determining humus in the soil. *Soil Sci.* 22: 221-232.

(21) Waksman, S. A., and Carey, C. 1926 The use of silica gel plate for demonstrating the occurrence and abundance of cellulose decomposing bacteria. *Jour. Bact.* 12: 87-95.

(22) Wells, B. W., and Shunk, I. V. 1928 A southern upland grass-sedge bog: ecological study. N. C. Agr. Exp. Sta. Tech. Bul. 32.

(23) White, J. W. 1914 Nitrification in relation to the reaction of the soil. *Penn. State Col. Ann. Rept.* 1914: 70-80.

(24) Willis, L. G. 1923 Nitrification and acidity in the muck soils of North Carolina. N. C. Agr. Exp. Sta. Tech. Bul. 24.

THE EFFECT OF MOISTURE CONTENT AND CROPPING ON EXCHANGEABLE CALCIUM AND MAGNESIUM, WITH PARTICULAR REFERENCE TO RICE SOIL[1]

W. H. METZGER[2]

Arkansas Agricultural Experiment Station

Received for publication January 14, 1929

INTRODUCTION

The subject of base exchange in soils has received considerable attention during the past five years. The nature of the exchangeable bases has been shown to have a very important bearing upon the physical properties of the soil. The effects of various factors which may influence the quantity and nature of the exchangeable bases have been investigated to a much less extent. Among these factors the influence of moisture content of the soil and of cropping upon the exchangeable bases has received little attention.

For several months the writer has been engaged in studies of soils utilized for the production of rice. The unusual moisture conditions under which rice is produced made it seem desirable to determine the effect of such conditions upon the exchangeable calcium and magnesium of the soil. The work reported in this paper was undertaken for the purpose of making more evident the effect of moisture content and cropping upon these exchangeable bases.

REVIEW OF LITERATURE

The literature of the subject has been rather extensively reviewed in a number of recent publications and an exhaustive review is, therefore, unnecessary here. All writers are not agreed upon the nature of the base exchange complex and the manner in which the exchangeable bases are held in the soil. One group believes that these bases are held in chemical combination, perhaps largely as complex alumino silicates, and that their replacement is accomplished by true chemical forces, subject to well defined chemical laws. In this group the works of Kelley and Brown (9), Burgess and McGeorge (2), and Kerr (10, 11) set forth some of the evidence supporting this view. Another group proposes that the exchangeable bases are adsorbed on the colloid particles of the soil. Hissink (6) advanced the supposition that the exchangeable cations

[1] Contribution from the Agronomy Department. Published with the approval of the Director of the Station. Research Paper No. 119, Journal Series, University of Arkansas.

[2] The thanks of the writer are hereby tendered to Mr. G. H. Banks, Assistant Director, In Charge of the Rice Branch Experiment Station at Stuttgart, Ark., who took the samples of Crowley silt loam and furnished the cropping history of this soil, and to Prof. C. K. McClelland for suggestions concerning the statistical treatment of the data.

are adsorbed as an external layer around the colloid particle, the inner layer being composed of the corresponding anions. This arrangement, in many respects, is similar to the Helmholtz double-layer, so familiar to students of colloid chemistry. Some recent publications by workers who apparently favor this view are those of Breazeale and Magistad (1) and Oden (16).

Some of the factors affecting the content of exchangeable bases of soils have been studied. Catherwood and DeTurk (3) have presented data showing the relation of maturity of soil type to exchangeable calcium and magnesium of various soil horizons. Humfeld (8) has shown the effect of fertilizer treatments upon the electrodialysable bases of the soil. Merkle (14) studied the effect of long-continued fertilizer treatments upon the exchangeable cations of the soil and found, in general, that fertilizers bearing the cations, Ca, Na, and K made significant increases in the amounts of these bases in exchangeable form. Ammonium sulfate treatment caused an increase in exchangeable hydrogen.

Martin (12) studied the effect of cropping (under conditions precluding leaching) upon the exchangeable bases of 13 California soils. He found continuous cropping for a period of twelve years, or cropping for two years with an intervening fallow period of 10 years, had no appreciable effect upon total exchangeable bases. Calcium and magnesium, comprising 90 per cent of the total exchangeable bases, were not changed in quantity. While it did not appreciably change the figures for total bases, the exchangeable potassium was decidedly reduced by two to twelve years of cropping with barley.

Ogg and Dow (17) found decidedly more exchangeable calcium in cultivated soils, as a group, than in "woodland," "hill or heath" soils, or soils "long unploughed."

DESCRIPTION OF SOILS

The rice soil investigated is classified as Crowley silt loam. It is the typical prairie soil of the rice section of Arkansas and is characterized by its very flat topography. The surface soil is a gray silt loam, of which silt comprises nearly 75 per cent and silt and clay together constitute 90 per cent. Underlying this at a depth of 20 to 24 inches is a very tight, highly plastic layer almost impervious to water, a very important feature of a soil well adapted to rice culture. Two horizons of this soil were studied, the first comprising the surface 7 inches and the second the 7 to 14 inches sub-surface layer. The reaction of the surface layer samples varied from pH 7.0 to 7.3, while the subsurface layer samples showed reactions varying from pH 6.5 to 6.9.

The soil used for the study of effect of moisture content and that used in the study of effect of cropping is classified as Clarksville silt loam. It contains four-fifths as much silt and clay as the Crowley silt loam, possesses a more open structure, and has no impervious layer in the subsoil. It is derived largely from non-magnesian limestone, and has been subjected to fairly heavy cropping

and rather extensive leaching for a long period of time. The reaction of the samples from this soil varied from pH 5.3 to 5.7.

METHODS

The method described by Hissink (7) was adopted with very slight modification for this work. Enough air-dry soil to constitute a 25-gram sample of water-free soil, finely pulverized, was treated in an Erlenmeyer flask with 100 cc. of N salt solution heated to 70°C. This was shaken occasionally and allowed to stand over night. The whole was then brought quantitatively onto the filter and leached with successive portions of salt solution at room temperature until one liter of leachate was secured.

Preliminary determinations showed that the amount of $CaCO_3$ in the soil samples was insufficient to introduce an appreciable error, a second liter of leachate in all cases containing only very small amounts of calcium. Ammonium chloride was used with the Crowley silt loam and sodium chloride of very high purity was used for all the Clarksville silt loam samples. Comparison of the two salts gave very good agreement in the results secured, the greater solvent action of the ammonium salt apparently largely offsetting the very small amount of calcium and magnesium in the sodium salt. Hence, no correction was made for the small amount of calcium and magnesium carried by the sodium salt.

Calcium was determined by the standard volumetric method and magnesium by a volumetric method described by Handy (5). This method for magnesium, though perhaps not quite so exact as the standard gravimetric method, gave satisfactory results and, requiring less time, enabled the writer to make a larger number of determinations than would have been possible otherwise.

EXCHANGEABLE CALCIUM AND MAGNESIUM IN AN OLD RICE SOIL (CROWLEY SILT LOAM)

The soil used in this study was taken from a number of plots of the Rice Branch Experiment Station at Stuttgart, Arkansas. Plots were selected which have never received fertilizer treatment, or at least have received none within the past 16 years. The samples were taken from soil which has been cropped to rice in all cases at least 15 of the past 18 years. The irrigation water used on all these plots comes from the same source, being from deep wells and containing considerable calcium and magnesium. The reaction of the irrigation water tested on two occasions during the last growing season was pH 7.3.

The results of the determinations on this soil are shown in table 1.

The results of table 1 are shown graphically in figure 1. The data differ from those for many soils in that the quantity of exchangeable calcium and magnesium is greater in the surface soil than in the underlying layer. The reason for this condition in the rice soil is undoubtedly the deposition of salts by the irrigation water in the upper stratum of the soil profile.

TABLE 1

Exchangeable calcium and magnesium in surface and subsurface horizons of a rice soil

SAMPLE NUMBER, SURFACE SOIL	EXCHANGEABLE CALCIUM		EXCHANGEABLE MAGNESIUM		RATIO OF M.E. Ca TO M.E. Mg	SAMPLE NUMBER, SUBSOIL	EXCHANGEABLE CALCIUM		EXCHANGEABLE MAGNESIUM		RATIO OF M.E. Ca TO M.E. Mg	DIFFERENCE IN Ca OF THE TWO HORIZONS	DIFFERENCE IN Mg OF THE TWO HORIZONS
	Per cent	M.E.*	Per cent	M.E.			Per cent	M.E.	Per cent	M.E.		*per cent*	*per cent*
101	0.0976	4.88	0.0254	2.09	2.33	201	0.0808	4.04	0.0182	1.50	2.69	0.0168	0.0072
102	0.1116	5.58	0.0259	2.13	2.62	202	0.0564	2.82	0.0110	0.90	3.13	0.0552	0.0149
104	0.1128	5.64	0.0220	1.81	3.11	204	0.0432	2.16	0.0096	0.79	2.73	0.0696	0.0124
105	0.1110	5.50	0.0264	2.17	2.53	205	0.0424	2.12	0.0081	0.67	3.16	0.0676	0.0183
106	0.1092	5.46	0.0225	1.85	2.95	206	0.0716	3.58	0.0206	1.69	2.11	0.0376	0.0019
107	0.1132	5.66	0.0220	1.81	3.12	207	0.0592	2.96	0.0096	0.79	3.74	0.0540	0.0124
15	0.1464	7.32	0.0355	2.91	2.51	18	0.0700	3.50	0.0192	1.57	2.23	0.0764	0.0163
16	0.1532	7.66	0.0350	2.87	2.66	19	0.0836	4.18	0.0220	1.80	2.32	0.0696	0.0130
17	0.1444	7.22	0.0292	2.40	3.00	20	0.0712	3.56	0.0177	1.45	2.45	0.0732	0.0115
21	0.1328	6.64	0.0321	2.64	2.51	26	0.1064	5.32	0.0244	2.00	2.66	0.0264	0.0077
24	0.1176	5.88	0.0316	2.59	2.27	28	0.1088	5.70	0.0273	2.24	2.54	0.0088	0.0043
25	0.1324	6.62	0.0321	2.64	2.50	29	0.1140	5.44	0.0250	2.05	2.65	0.0184	0.0071
Mean†....	0.1234	6.17	0.0283	2.33	2.68	...	0.0756	3.78	0.0177	1.45	2.70	0.0478	0.0106
S D.......	0.0169	....	0.0047	....	0.089	...	0.0232	....	0.00607	....	0.316	......	......
C V......	13.6	....	16.6	....	3.32	...	30.6	....	34.2	....	11.7	......	......
Odds........												Infinite	Infinite

* M.E. = milligram equivalents per 100 gm. of soil.

† Formulae used in statistical analysis were as follows:

$$S\,D = \sqrt{\frac{\Sigma D^2}{N}}.$$

$$C\,V \text{ (coefficient of variability)} = \frac{100 \times S\,D}{\text{Mean}}.$$

$$P\,E\,(M) \text{ (probable error of the mean)} = \frac{.6745 \times S\,D}{\sqrt{N}}.$$

$$P\,E \text{ (difference)} = \sqrt{E^2_a + E^2_b}.$$

$$\text{Odds (student's method using Love's tables) } Z = \frac{\text{Mean (difference)}}{S\,D}.$$

The soil of the area from which these samples were taken appears to be extraordinarily uniform. The figures, on the contrary, indicate that there is probably considerable difference in the amount of leaching taking place, as the coefficient of variability for the subsurface samples in case of both bases, is

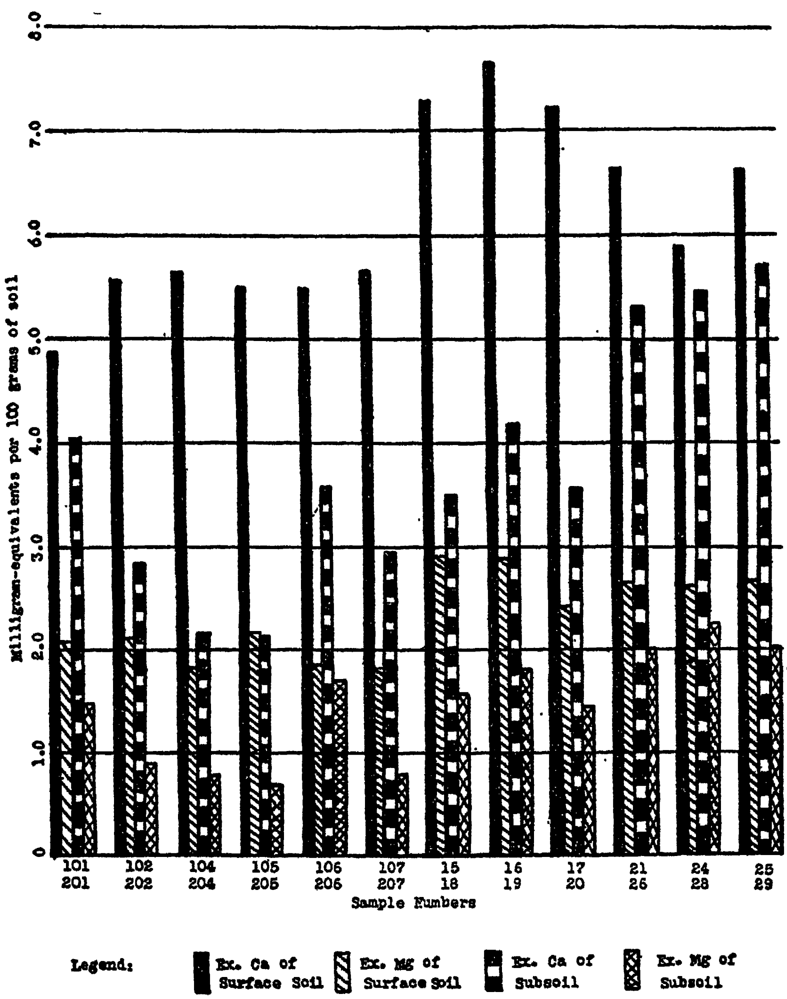


FIG. 1. EXCHANGEABLE CALCIUM AND MAGNESIUM IN TWELVE SAMPLES FROM EACH OF TWO HORIZONS OF AN OLD RICE SOIL

rather high. The impervious nature of the subsoil in general, however, is indicated by the relatively low content of exchangeable calcium and magnesium in most of the samples from the sub-surface horizon. The ratio of calcium to magnesium is rather remarkably constant throughout.

EFFECT OF MOISTURE CONTENT OF THE SOIL UPON THE EXCHANGEABLE CALCIUM AND MAGNESIUM

The ordinary procedure in rice production is to grow the crop in standing water for a period of about 75 days, beginning within 10 to 20 days from the time the plants emerge from the soil. Therefore, it seemed desirable to determine what effect submergence might have upon the exchangeable calcium and magnesium of the soil. This problem could not be studied through the use of field samples because such samples are modified by the salts carried in the irrigation water. Accordingly, a thoroughly mixed, screened and finely pulverized quantity of Clarksville silt loam was divided into 30 samples of 200 gm. each for laboratory experimentation. These samples were placed in wide-mouthed bottles and distilled water added to ten of them until it stood ½ inch above the surface of the soil. A second group of 10 samples received sufficient distilled water to bring the moisture content to 20 per cent, which is approximately optimum for the soil. The third group of 10 samples was left air-dry. The bottles were then weighed and stored where they were not exposed to the air of the laboratory. Sufficient distilled water to bring the samples up to weight was added once each week. After 75 days no more water was added and the soil was allowed to become air-dry. It was then finely pulverized and subjected to analysis. The results are listed in table 2.

When the results of these determinations are compared one group with another, some very interesting facts are revealed. Considering calcium first, the means of the columns for the air-dry and 20 per cent moisture samples show a difference of only 0.0002 $\pm$0.00045 per cent, entirely insignificant. When the mean of the air-dry samples or of the 20 per cent moisture samples (the two being practically the same) is compared with the mean of the flooded samples, a difference of 0.0104 $\pm$0.00047 per cent is obtained. This difference is 22.1 times the probable error and, hence, gives very high odds that it is significant. Thus, flooding the soil for a period of 75 days depressed the exchangeable calcium.

Now turning to magnesium and comparing the mean of the air-dry samples with that of the 20 per cent moisture samples, a difference of 0.0012 $\pm$ 0.00021 per cent is obtained. This difference is 5.7 times the probable error and established a reasonably high degree of probability that 20 per cent moisture content caused a significant increase in exchangeable magnesium. Comparing air-dry samples with flooded ones a difference of 0.0102 $\pm$ 0.000435 per cent is obtained, 23.4 times the probable error. The 20 per cent moisture samples compared with the flooded ones show a difference of 0.0090 $\pm$ 0.000435 per cent in favor of the flooded ones. This is 20.6 times the probable error. Thus, it is apparent that flooding had opposite effects upon the exchangeable calcium and magnesium, depressing the quantity of the former and increasing the latter. The ratio between the two, therefore, is very decidedly narrowed. These results are shown graphically in figure 2.

TABLE 2

Exchangeable calcium and magnesium in Clarksville silt loam kept at various moisture contents for a period of seventy five days

Sample number	AIR-DRY SOIL					20 PER CENT MOISTURE						FLOODED					
	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg	Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg	Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg
	Per cent	M.E.	Per cent	M.E.			Per cent	M.E.	Per cent	M.E.			Per cent	M.E.	Per cent	M.E.	
D1	0.0924	4.62	0.0086	0.71	5.79	1–20	0.0952	4.76	0.0096	0.79	4.85	1S	0.0832	4.16	0.0201	1.65	2.18
D2	0.0912	4.56	0.0091	0.74	6.33	2–20	0.0924	4.62	0.0120	0.98	5.91	2S	0.0808	4.04	0.0206	1.69	2.56
D3	0.0900	4.50	0.0086	0.71	6.16	3–20	0.0920	4.60	0.0108	0.89	5.59	3S	0.0808	4.04	0.0201	1.65	2.37
D4	0.0916	4.58	0.0096	0.79	6.42	4–20	0.0932	4.67	0.0096	0.79	5.21	4S	0.0824	4.12	0.0158	1.30	2.85
D5	0.0912	4.56	0.0086	0.71	6.50	5–20	0.0928	4.64	0.0108	0.89	5.21	5S	0.0804	4.02	0.0192	1.57	2.82
D6	0.0920	4.60	0.0100	0.82	5.60	6–20	0.0908	4.54	0.0105	0.87	5.16	6S	0.0808	4.04	0.0225	1.85	2.36
D7	0.0908	4.52	0.0091	0.74	6.10	7–20	0.0884	4.42	0.0108	0.89	4.71	7S	0.0804	4.02	0.0172	1.41	3.16
D8	0.0908	4.52	0.0091	0.74	6.10	8–20	0.0884	4.42	0.0096	0.79	5.04	8S	0.0800	4.00	0.0206	1.69	2.39
D9	0.0912	4.56	0.0091	0.74	6.10	9–20	0.0908	4.54	0.0110	0.90	5.28	9S	0.0820	4.10	0.0211	1.73	2.44
D10	0.0924	4.62	0.0110	0.90	6.16	10–20	0.0920	4.60	0.0105	0.87	6.02	10S	0.0812	4.06	0.0177	1.44	2.52
Mean........	0.0914	4.56	0.0093	0.76	6.03	.....	0.0916	4.58	0.0105	0.87	5.30	.....	0.0812	4.06	0.0195	1.59	2.57
S D..........	0.0007	....	0.0007	....	0.394	.....	0.00199	....	0.0007	....	0.403	.....	0.00096	....	0.00192	....	0.243
P E (M)......	±0.000149	....	±0.000149	....	±0.0841	.....	±0.000424	....	±0.000149	....	±0.08602	.....	±0.000204	....	±0.000409	....	±0.0518
C V..........	0.76	....	7.52	....	6.53	.....	2.17	....	6.66	....	7.60	.....	1.18	....	9.84	....	9.45

In order to further verify the results set forth in the preceding paragraphs, a group of soil samples was taken from jars in the greenhouse in which various rice experiments had just been completed. Clarksville silt loam had been used in these experiments. Four pairs of jars were sampled, each consisting of one jar in which the soil had been flooded and one in which similar soil was kept at optimum moisture content. Comparisons between jars of one pair and those of another were not possible, as the soil of the various pairs of jars

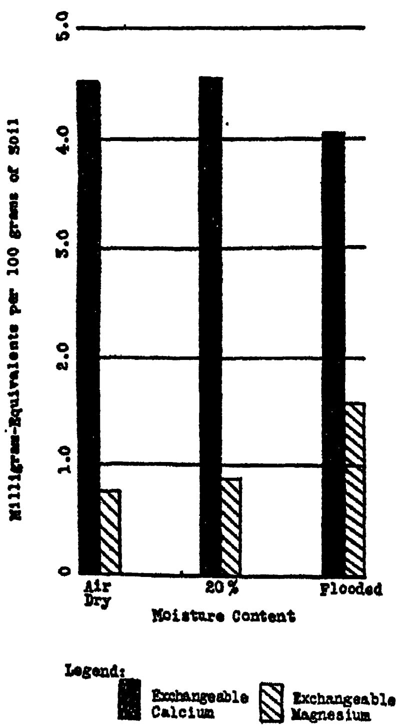


FIG. 2. EFFECT OF MOISTURE CONTENT UPON EXCHANGEABLE CALCIUM AND MAGNESIUM
(Average of ten samples)

was not from the same source. The result of the anlayses for exchangeable calcium and magnesium in these samples are given in table 3.

The results with the greenhouse soil samples show the same general effects of flooding, calcium being depressed and magnesium increased. There is one exception in the figures for magnesium, the sample of soil kept at optimum moisture content in one case showing more exchangeable magnesium than the flooded soil. The writer can offer no explanation for this exception.

THE EFFECT OF ONE SEASON OF CROPPING WITH CORN UPON EXCHANGEABLE CALCIUM AND MAGNESIUM

It would have been desirable in this work to have studied the effect of cropping with rice upon these bases, but material for such a study was not available. Rice does not require large amounts of calcium and since corn also does not draw heavily upon the calcium and magnesium of the soil, the effect of cropping with corn was studied. Cropped and fallowed plots with no fertilizer treatment were available for sampling. Nine pairs of such plots were sampled thoroughly and the composite sample from each plot mixed well and analyzed. The results are shown in table 4.

TABLE 3

Exchangeable calcium and magnesium in samples of greenhouse soil, flooded, and kept at optimum moisture content

SAMPLE NUMBER	TREATMENT	EXCHANGEABLE CALCIUM		EXCHANGEABLE MAGNESIUM		RATIO OF M.E. Ca TO M.E. Mg
		Per cent	M.E.	Per cent	M.E.	
278	No crop, flooded	0.1128	5.64	0.0235	1.93	2.92
288	No crop, optimum moisture	0.1216	6.08	0.0137	1.12	5.42
279	No crop, flooded	0.1129	5.64	0.0152	1.25	4.51
289	No crop, optimum moisture	0.1211	6.05	0.0126	1.03	5.87
234	Cropped,* flooded	0.0364	1.82	0.0104	0.85	2.14
238	Cropped,* optimum moisture	0.0550	2.75	0.0099	0.81	3.39
44	Cropped, flooded	0.0285	1.42	0.0104	0.85	1.67
46	Cropped, optimum moisture	0.0336	1.68	0.0126	1.03	1.53

* Cropped twice, successively with rice.

These results do not indicate any measurable effect of cropping with corn for one season, as compared to fallowing, upon the exchangeable calcium and magnesium. The mean differences obtained are supported by insignificant odds. To add further evidence on this point, however, a pair of plots was selected which had been subject to a certain amount of what appeared to be very uniform sheet erosion. These were selected because it was believed they would be low in exchangeable calcium and magnesium and thus small differences might be more readily measured. Ten samples were removed from the plot which was maturing a good crop of corn and 10 from the fallowed plot. The results secured with these samples are given in table 5.

When the results for calcium in table 5 are compared, the difference of the means is found to be 0.0025 ± 0.0079 per cent. The difference is, therefore, less than the probable error and hence, not significant. Comparing the results for magnesium, a difference of the means of 0.001 ± 0.00056 per cent is found,

TABLE 4

Exchangeable calcium and magnesium of nine cropped and nine fallowed plots of Clarksville silt loam

FALLOWED SOIL						CROPPED SOIL						DIFFERENCES IN THE TWO AREAS	
Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg	Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg	In per cent of calcium	In per cent of magnesium
	Per cent	M.E.	Per cent	M.E.			Per cent	M.E.	Per cent	M.E.			
2	0.0828	4.14	0.0057	0.46	9.00	1	0.0976	4.88	0.0057	0.46	10.60	−0.0148	0.0000
3	0.0700	3.50	0.0060	0.49	7.14	4	0.0688	3.44	0.0062	0.50	6.68	+0.0012	−0.0002
6	0.0892	4.46	0.0081	0.66	6.75	5	0.0820	4.10	0.0076	0.62	6.61	+0.0072	+0.0005
7	0.0800	4.00	0.0053	0.43	9.30	8	0.1008	5.04	0.0062	0.50	10.08	−0.0208	−0.0009
10	0.0814	4.07	0.0067	0.55	7.40	9	0.0948	4.74	0.0076	0.62	7.64	−0.0134	−0.0009
11	0.0788	3.94	0.0091	0.74	5.32	12	0.0580	2.90	0.0096	0.78	3.72	+0.0208	−0.0005
14	0.0968	4.84	0.0103	0.84	5.76	13	0.0912	4.56	0.0105	0.86	5.30	+0.0056	−0.0002
18	0.0836	4.18	0.0088	0.72	5.80	17	0.0928	4.64	0.0088	0.72	6.44	−0.0092	0.0000
19	0.0928	4.64	0.0081	0.66	7.03	20	0.0988	4.94	0.0062	0.50	9.88	−0.0060	+0.0019
Mean.....	0.0839	4.19	0.0075	0.61	7.05	..	0.0872	4.36	0.0076	0.62	7.44	−0.0029	−0.00003
S D.......	0.0075	....	0.0016	....	1.30	..	0.0139	....	0.0016	0.01	2.20	....	....
C V.......	8.93	....	21.3	....	18.4	..	15.9	....	21.0	0.62	29.4	....	....
Odds.........												3.19 to 1	None

TABLE 5

Exchangeable calcium and magnesium in a fallowed plot and a plot cropped to corn for one season

FALLOWED PLOT						CROPPED PLOT					
Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg	Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg
	Per cent	M.E.	Per cent	M.E.			Per cent	M.E.	Per cent	M.E.	
F1	0.0836	4.18	0.0096	0.78	5.35	C1	0.0688	3.44	0.0091	0.74	4.64
F2	0.1056	5.28	0.0086	0.70	7.54	C2	0.0936	4.68	0.0096	0.78	6.00
F3	0.0460	2.30	0.0081	0.66	3.47	C3	0.0548	2.74	0.0076	0.62	4.41
F4	0.0420	2.10	0.0052	0.42	5.00	C4	0.0360	1.80	0.0062	0.50	3.60
F5	0.0272	1.36	0.0062	0.50	2.72	C5	0.0336	1.68	0.0086	0.70	2.40
F6	0.0880	4.40	0.0072	0.59	7.45	C6	0.1128	5.64	0.0128	1.05	5.37
F7	0.0360	1.80	0.0052	0.42	4.28	C7	0.0404	2.02	0.0057	0.46	4.34
F8	0.0404	2.02	0.0062	0.50	4.04	C8	0.0380	1.90	0.0076	0.62	3.06
F9	0.0680	3.40	0.0057	0.46	7.39	C9	0.0412	2.06	0.0048	0.39	5.28
F10	0.0360	1.80	0.0052	0.42	4.28	C10	0.0280	1.40	0.0057	0.46	3.04
Mean........	0.0572	2.86	0.0067	0.54	5.15	....	0.0547	2.73	0.0077	0.63	4.21
S D...........	0.0256	....	0.00149	....	1.66	....	0.0269	....	0.00226	....	1.108
P E (M).......	±0.00546	....	±0.000318	....	±0.354	....	±0.0057	....	±0.00046	....	±0.236
C V...........	44.7	....	22.2	....	32.2	....	49.1	....	29.3	....	26.3

favoring the cropped soil. This difference, however, is less than twice the probable error and it, also, is not significant. These results, therefore, are in accord with those previously presented and indicate that one season of cropping with corn produced no measurable effect upon the exchangeable calcium and magnesium of the soil. The selection of plots for this study prove a rather unfortunate one as both plots, though only 1/20-acre in size, showed high variability.

DISCUSSION OF RESULTS PRESENTED

The figures obtained for the old rice soil, Crowley silt loam, reveal no unusual characteristics of the soil, except the rather infrequently encountered situation in which the greater concentration of the exchangeable bases is found near the surface. The deposition of salts by the irrigation water in the surface soil due to restricted leaching, is responsible for this condition.

The explanation for the depressing effect upon the exchangeable calcium produced by flooding the soil and its building-up effect upon the exchangeable magnesium is much more difficult. It has been shown by Subrahmanyan (18) that keeping a soil saturated with water reduces its hydrogen-ion concentration. He presented data, also, which showed that the decrease in hydrogen-ion concentration was concomitant with the increase in free and saline ammonia. The writer, working with Janssen (15) also observed this fact in rice soils, and found that most of the decrease in hydrogen-ion concentration disappeared when the soil was dried for a few days previous to sampling. It is rather difficult to see how this change could appreciably affect the exchangeable calcium and magnesium, particularly in divergent manner. If the flood water had been decanted or the soil solution displaced before the samples were analyzed, the decrease in calcium might be attributed, in part at least, to passage of a portion of the exchangeable calcium into solution. This would still leave the increase in exchangeable magnesium to be explained. The flood water was allowed to dry in the soil, however, and the soil solution was not displaced before the samples were treated with the salt solution. Gedroiz (4) found a very small increase in exchangeable calcium in soil which stood submerged in *n* NaCl solution for 3 months and for 14 months, but this, of course, represents very different conditions than flooding with distilled water. Martin and Christie (13) waterlogged a sandy loam soil and found that the concentration of calcium and of magnesium in the soil solution was depressed.

The writer is unable to offer an explanation for the divergent behavior of these bases in flooded soil. It would seem, however, that the phenomenon might be more easily explained if it is assumed that the exchangeable bases are held in the soil by true chemical forces, rather than to assume the double-layer type of adsorption proposed by Hissink. Submergence affected the soil colloids, and it required nearly one-third more time to leach the flooded soil samples than the corresponding samples kept air-dry or at optimum moisture content. Such a change in the physical condition of the soil might be advanced

to explain the decrease in exchangeable calcium, but it is difficult to see how the increase in magnesium could be explained on this basis.

Considering the fact that a corn crop which would yield 60 bushels per acre would remove perhaps not more than 2 per cent of the amount of calcium held in exchangeable form in the soil studied, (and in many cases, probable, less than 2 per cent) it is not surprising that no effect of cropping could be measured. The results are in accord with those secured by Martin (12), though his results are of greater significance because the periods of cropping and fallowing are much longer.

An interesting feature of the results secured with Clarksville silt loam is the rather wide ratio of calcium to magnesium. Most of the soils of this country upon which reports have been published show ratios approaching more nearly that of the Crowley silt loam.

SUMMARY

The results of a study of the exchangeable calcium and magnesium of a soil cropped to rice for a long period of time are reported in the foregoing paragraphs. The effect of flooding a soil, as in rice fields, and the effect of a season of cropping with corn upon the exchangeable calcium and magnesium were also studied.

Crowley silt loam cropped to rice for 15 of the past 20 years showed considerably more exchangeable calcium and magnesium in the surface soil than in the sub-surface horizon.

Keeping Clarksville silt loam at a moisture content of 20 per cent with distilled water for a period of 75 days increased the exchangeable magnesium as compared to the same soil kept air-dry. Exchangeable calcium was not appreciably changed. Flooding with distilled water for the same period of time depressed the amount of exchangeable calcium, but increased the amount of exchangeable magnesium.

Cropping with corn for one season brought about no measurable change, when compared with fallowing, upon the exchangeable calcium and magnesium of Clarksville silt loam.

REFERENCES

(1) Breazeale, J. F., and Magistad, O. C. 1928 Base exchange in orthoclase. Arizona Sta. Tech. Bul. 24.

(2) Burgess, P. S., and McGeorge, W. T. 1927 Zeolite formation and base-exchange reactions in soils. Arizona Tech. Bul. 15.

(3) Catherwood, M. P., and DeTurk, E. E. 1928 The relation of soil type to the exchangeable calcium and magnesium in some Illinois soils. *Jour. Amer. Soc. Agron.* 20: 7: 657–678.

(4) Gedroiz, K. K. 1913 Colloidal chemistry as related to soil science. II. Rapidity of reaction exchange in the soil. Translation by Waksman, mimeographed and distributed by United States Department of Agriculture, Vol. 1.

(5) Handy, J. O. 1900 The volumetric determination of magnesia. *Jour. Amer. Chem. Soc.* 22: 31–39.

(6) HISSINK, D. J. 1924 Base exchange in soils. *Trans. Faraday Soc.* 20: 551–566.
(7) HISSINK, D. J. 1923 Method for estimating adsorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.
(8) HUMFELD, HARRY 1928 Electrodialysis of soils: III. Effect of different fertilizer treatments of soils on the bases and acids extracted by electrodialysis. *Jour. Amer. Soc. Agron.* 20: 11: 1141–1159.
(9) KELLEY, W. P., AND BROWN, S. MELVIN 1924 Replaceable bases in soils. Cal. Sta. Tech. Paper No. 15.
(10) KERR, H. W. 1928 The nature of base exchange and soil acidity. *Jour. Amer. Soc. Agron.* 20: 4: 309–335.
(11) KERR, H. W. 1928 The identification and composition of the soil alumino silicate active in base exchange and soil acidity. *Soil Sci.* 26: 5: 385–398.
(12) MARTIN, J. C. 1928 Private communication.
(13) MARTIN, J. C., AND CHRISTIE, A. W. 1919 Effect of variation in moisture content on the water-extractable matter of soils. *Jour. Agr. Res.* 18: 3: 139–143.
(14) MERKLE, F. G. 1928 The influence of fertilizer treatments on the content of exchangeable cations in Hagerstown silt loam. *Soil Sci.* 26: 5: 377–384.
(15) METZGER, W. H., AND JANSSEN, GEORGE 1928 The relation of sodium nitrate and certain other nitrogen carriers to the development of chlorosis in rice. *Jour. Agr. Res.* 37: 10: 589–602.
(16) ODEN, SVEN 1927 Om Jordens Surhetsgrad, Bufferverkan och Kalkbehov. Meddelande No. 318 fra Centralanstalten för forsöksväsendet pa Jordbruksomradet. (Citation by Breazeale, J. F., and Magistad, O. C., "Base Exchange in Orthoclase," Arizona Sta. Tech. Bul. 24, 1928. Original not seen.)
(17) OGG, W. G., AND DOW, W. T. 1928 The reaction, exchangeable calcium, and lime requirement of certain Scottish soils. *Jour. Agr. Sci.* 18, Part 1, pp. 131–157.
(18) SUBRAHMANYAN, V. 1927 Biochemistry of water-logged soils. Part I. The effect of water-logging on the different forms of nitrogen, on the reaction, on the gaseous relationships, and on the bacterial flora. *Jour. Agr. Sci.* 17: 429–448.

SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: I. HISTORICAL AND INTRODUCTORY

ROBERT L. STARKEY

New Jersey Agricultural Experiment Station

Received for publication January 22, 1929

The influences which higher plants may exert upon development of the microscopic population of the soil and the effects which such microörganisms may have are naturally numerous. The fact that plant growth continues in soil and that microörganisms develop in this habitat are ample verification of this fact. Neither group of organisms can develop long in the absence of the other in nature without showing abnormalities due to the absence of the other associate. Most of the soil organisms depend upon the organic and inorganic plant residues as sources of food and higher plants depend upon the conversion of their own remains by microörganisms into assimilable substances as sources of nutrients. Inhibition of activities of either group of organisms eventually leads to modified development of the other. The reciprocal effects of the development of higher plants in intimate relationship with microörganisms are associated with a multitude of reactions occurring in the soil wherever higher plants grow. The activities associated with the complex transformation of organic and inorganic substances are largely regulated by microbial development. Starting with large mineral aggregates with which plant residues are incorporated the microörganisms play very important rôles in developing a disintegrated admixture of fine particles capable of supporting higher plants and furnishing to them the nutrients, either inorganic or organic, which are necessary for their maturity.

The plants withdraw considerable amounts of substances (principally inorganic) from the rhizosphere, modify the soil structure by the penetration of roots, exert solvent effects by the excretion of carbon dioxide and eventually introduce large amounts of organic matter to the soil in the form of their dead tissues which undergo disintegration through the agency of the microörganisms. These changes all affect the development of the soil organisms. In a comprehensive way it is difficult to distinguish between those activities of plant growth which do and do not affect microbial development and equally difficult to determine what activities of microörganisms in soils do not exert direct or indirect effects upon plant growth.

Close to the root systems the reactions of soil microörganisms may not be qualitatively different from those at a distance from the roots, but they may, and undoubtedly do, differ quantitatively due to the influences of the higher

plants and further, their effects upon plant development become apparent more quickly since their action occurs nearer to the organs of absorption of the higher plants.

HISTORICAL

Results of studies concerned with the influences of development of higher plants upon the abundance of groups of microörganisms or microbial activities in soils about root systems may be interpreted as reflecting the resultant effect of a variety of influences of the plants upon the rhizosphere, partly depressive and partly accelerative. Among the various modifications of the soil which the plants may provoke are the following: lowering of the concentration of certain mineral nutrients in the soil due to their absorption, partial desiccation of the soil by absorption of water, increase in soil carbonates following root excretion of carbon dioxide, contribution to microbial foods by sloughed off root portions and excretions. Some of these effects may directly affect the soil population and others indirectly by modifications of the physical composition of the soil. The attraction of phytophagous insects and worms about roots may eventually lead to influences upon the microörganisms.

Knowing what marked changes are brought about in soils by desiccation (26, 23) and what influences such effects may exert upon the biological properties (67), it is not surprising that combinations of a variety of environmental changes should result in modifications of the biological activities.

Rokitskaia (51) observed that decomposition of cellulose proceeded at different rates in soils obtained from the root zones of various plants. The explanation of this effect may appear from an appreciation of the nutrition of the soil organisms which naturally require considerable abundance of mineral nutrients and available nitrogen to decompose cellulose. The different degrees of exhaustion of the nitrogen and other necessary substances by root absorption may explain the observed effects on cellulose decomposition.

Microörganisms penetrating the plant. The most striking development of microörganisms about roots of higher plants appear in cases of actual penetration of roots by the microörganisms. This may result in pathological conditions where the microörganisms cause marked destruction of cells and tissues; it may result in passive conditions with little or no injury; or it may be a condition of actual beneficial effects of the invader upon the host. Several classes of this last case are known: 1) the association of bacteria with legumes, 2) mycorrhiza formations with such plants as woody trees, orchids and heathers (49, 40), 3) possible cases of stimulation to root development (50), 4) invasion of many non-legumes by passive or symbiotic bacteria called "bacteriorrhiza" by Perotti (47, see also 57, pages 447–460).

Penetration of roots of most plants including annuals and perennials, woody and herbaceous is probably the rule rather than the exception (20). The extent of the penetration and degree of the effects vary with the soil conditions which determine the vigor and degree of resistance. Under favorable cultural

conditions there may be no apparent injury but, under adverse conditions of moisture, temperature, or nutrient supply the invader may caused definite injury.

Influence of inorganic root excretions upon soil organisms. Excretion of both organic and inorganic substances by plant roots appear of particular interest in their influences upon microbial activities as well as in nutrition of the plants themselves. Although it is apparent that higher plants must absorb more of the inorganic substances than they excrete, still at certain stages of growth, there may be appreciable elimination of certain substances both organic and inorganic, nitrogenous and non-nitrogenous.

Fred and Haas (13) verified the observations of Sachs (52) and Knop (24) that roots may exert a solvent effect upon limestone by the action of the carbon dioxide which is liberated. They further extended these observations by demonstrating that the presence of microörganisms enhanced this solvent action. This suggests that the microörganisms may exert pronounced effects about roots as a result of their ability to produce carbon dioxide presumably from the decomposition of organic root excretions. Stoklasa and Ernest (58, 59) and Aberson (1) showed that the strong corrosive action was due to carbon dioxide and that other acids played no important rôle under normal conditions of plant growth. Czapek (11) and Wilfarth, Römer and Wimmer (71) observed that appreciable amounts of inorganic compounds of potassium, calcium, magnesium, phosphates, sulfates, and chlorides may be eliminated by the plant roots into the soil. Stoklasa (58, 59, 55), Kunze (25) and others (see 41, 17) have shown that the carbon dioxide evolved from roots and by microörganisms greatly facilitates absorption of essential elements from relatively insoluble soil minerals. Newton (43, 44) emphasizes the importance of excretion of carbon dioxide by plant roots in the feeding power of plants. The results of Parker (45, 46) show that there may be no correlation between amounts of nutrients absorbed and carbon dioxide evolved by the roots of higher plants growing in the presence of an abundance of soluble nutrients (as in cultural solutions). However, in the soil environment, the carbon dioxide evolved from roots and by the soil organisms may play an important rôle in determining the ability of higher plants to absorb nutrients. The consumption of oxygen and formation of carbon dioxide in the soil both from microbial action and from plant roots may exert pronounced effects upon root development (7, 8).

Neller (42) observed that the carbon dioxide produced from soil supporting growth of higher plants was appreciably greater than the amounts produced by unplanted soil. In explaining his results it seems necessary to assume that the plant growth actually must have accelerated the decomposition of the soil organic matter by effecting a more thorough aeration or some other similar action. Lundegardh (30) concluded that the contribution of the microörganisms to the carbon dioxide of the atmosphere generally far exceeds that of the roots of higher plants under normal conditions. Stoklasa's results

(57) would lead to a similar conclusion. Barakov (2) and Turpin (62) concluded that the plant often produces, at the period of its most active growth, many times as much carbon dioxide as is produced by soil organisms, and that the excess of carbon dioxide in the soil growing a crop is due to respiratory activity of the plants rather than to the decay of root particles from the crop growing upon the soil. That this conclusion may not be entirely justified may appear from the experimental results to be reported. Metzger (41) found the soil bicarbonates in much greater concentration near plant roots than at a distance from the roots. The greatest increase in bicarbonates appeared at the period of heading of the plants. What portion of these effects may be attributed to the soil microörganisms is speculative.

Headden (17) also observed a marked increase in carbon dioxide in soils under growing plants and attributed to a large degree the beneficial influence of one crop upon subsequent ones to the solvent action which this carbon dioxide exerts upon insoluble soil minerals. Due to the fact that the amount of carbon dioxide in the soil decreased to a low level following the cutting of a crop it was concluded that the growth of higher plants suppresses microbial activities and that the increase in carbon dioxide found under plants is due to excretion of the gas from the plant roots. These conclusions are not in accord with facts observed by others regarding the development of microorganisms in the rhizosphere (21, 27, 68, 29, 53, 16).

Influence of organic root excretions upon soil organisms. Little is known as to the nature or abundance of excretions of organic substances from roots. Lyon and Wilson (35) determined the amounts of residues and excretions from plant roots developing in solutions free from microörganisms. These solutions showed the presence of organic nitrogen even before the nitrate nitrogen had all been absorbed. In some cases there appeared to be a decrease in the amount of organic nitrogen with progressive stages in the life of the plant, especially at the period close to maturity. The ratio of organic matter in solution to that in the plant was from 1–37 to 1–65. The organic matter contained relatively small amounts of nitrogen. Cranner (9) found a considerable abundance of phosphatides excreted by roots of higher plants, seeds and seedlings. It was believed by Melin (39) that such materials play an important rôle in favoring development of mycorrhiza fungi about roots leading to their subsequent penetration of the roots and associative development with the plants.

The much discussed problem of soil toxins, and effects of one drop upon another may find interpretation from information concerning root excretions, absorption by roots and residual products of plant disintegration. Associative effects of plants and microörganisms find expression in the modification of the abundance of cells of different physiologic groups of microörganisms about roots and their activity in the rhizosphere. Beijerinck (4) found larger numbers of Azotobacter in soil close to roots of legumes than in soil from roots of non-legumes. It may be logical to consider that organic plant

residues about plant roots would serve as a suitable source of food for nitrogen fixing organisms consequently favoring their development and associated fixation of nitrogen. In fact, Truffaut and Bezssonov (61) claim to have grown corn to maturity in media containing no nitrogen but containing nitrogen-fixing bacteria. It was believed that the organic matter excreted from the plant roots served as food for the bacteria, which fixed the nitrogen, which in turn became available to the plants. Such results as these should be repeatedly verified before being seriously accepted as indicating the facts. It seems unlikely, however, even though small amounts of nitrogen were fixed under such conditions, that they would be sufficient to satisfy the demands of the plants. Further, since the nitrogen-fixing bacteria were the only ones present, it seems unlikely that any appreciable amount of the fixed nitrogen would become available to the plants. At least, it is known that, under natural conditions where the same agencies should appear active,

TABLE 1

Influence of plant development upon abundance of bacteria and formation of carbon dioxide (Stoklasa)

SOIL DEPTH	PASTURE		LUCERNE		WHEAT		SUGAR BEETS	
	Bacteria	CO_2	Bacteria	CO_2	Bacteria	CO_2	Bacteria	CO_2
cm.	*millions*	*mgm.**	*millions*	*mgm.**	*millions*	*mgm.**	*millions*	*mgm.**
10–20	2.300	16.5	54.800	60.6	24.000	47.5	38.700	56.4
20–30	2.500	19.4	52.500	62.8	25.600	49.7	41.200	58.2
30–50	0.140	9.8	14.800	46.2	26.000	28.5	22.000	36.4
50–80	0.012	3.3	0.770	16.3	2.200	6.6	5.500	8.3
80–100	0.005	2.2	0.082	3.7	0.049	4.3	0.200	4.8

* From 1 kg. soil in 24 hours.

non-legumes require the presence of an abundance of nitrogen which is not supplied through the agency of non-symbiotic nitrogen-fixing organisms.

Joshi (21) showed that non-legumes might benefit from associative development of nitrogen-fixing bacteria although no nodulation developed.

Influence of plant development upon abundance of soil microörganisms. Velich (63) found an organism called *Clostridium gelatinosum*, Laxa. in greater abundance in soils close to the roots and upon the root epidermis of beets, mangels, barley, rye, oats and wheat than at a distance from the roots. Similar observations were also made by Maassen and Gruber (see Löhnis, 28, p. 16). Hoffman (18) reported that, in 27 out of 32 cases investigated, the numbers of bacteria in soils adjacent to roots were much greater than in soils at a distance from the growing roots. Further studies demonstrated that development of different plants exerts different effects upon any one organism and that development of different organisms may exert a variety of effects upon any one plant.

LeClair (27) found the bacteria more abundant in planted than in un-

planted soil. Johnson and Levine (19) found certain gram negative bacteria (coli-like) in greater numbers in planted soils. Greaves, Stewart and Hirst (61) found the numbers of organisms more abundant in cropped than in fallow soil. Stoklasa's results (56) show a rather close correlation between the abundance of bacteria in soils under different plants and the evolution of carbon dioxide from these soils. Further, these differences were apparent not only in the surface layers of soil but persisted even in the deep layers (table 1). The fact that roots of weeds evolved greater amounts of carbon dioxide than cultivated plants led him to believe that such plants had a greater feeding power and consequently could frequently compete to advantage with cultivated plants.

Somewhat larger numbers of nitrogen-fixing bacteria and total bacteria were found in planted than unplanted soils by Joshi (21). Legumes favored the development of the greatest abundance of organisms.

Wilson and Lyon (68) grew plants under controlled conditions in sterilized soils inoculated with pure cultures of bacteria with the results that much greater numbers of bacteria developed in practically all cases as compared with development in the absence of plant growth.

The results of Löhnis (29) show that the numbers of bacteria increased as a result of plant growth, particularly in the case of some of the legumes. The response to the crops was much greater the second year. This might be expected in view of the fact that continuous cropping leaves residues which should keep the abundance of organisms at a relatively high level; an uncropped soil, however, undergoes progressive depletion of microbial foods and consequently decreases in biological activity. The numbers of *B. radiobacter* fluctuated, but in general were more numerous under the legumes. The actinomyces and filamentous fungi were slightly higher under plants than in the absence of plant growth. The general increases in the soil population persisted even after the periods of harvesting the crops but a thorough desiccation of the soil caused a more or less complete disappearance of the effects.

Creuzburg (10) attacked the problem in the identical way followed by Löhnis but obtained less striking results. Although some increase in numbers of bacteria was observed under legumes, even a decrease was found under cereals. Even second plantings of crops gave very slight differences under cereals. He concluded that only certain plants under certain conditions exert an influence upon numbers of soil bacteria. No significant influence of the stage of growth of the plants upon the abundance of organisms was apparent from the results of either Löhnis or Creuzburg.

Smith (53) observed that growth of legumes increased the numbers of *B. radiobacter* from the third week after planting and this effect persisted some weeks after harvesting the crops. Although the results in the field were less striking than in the greenhouse, the abundance of these organisms was greater nearer the roots, often none being found one foot distant from the roots.

Taranovskaia and Germanov (60) believed that the increases in numbers

of bacteria and acceleration of nitrate formation by growth of clover was due to improvement in the physical structure and physico-chemical properties of the soil by plant growth. From the results of Batchelor and Curie (3) there appears to be a correlation between crop yields (and consequently root residues) and abundance of nitrogen-fixing anaerobes in soils. The greatest number of the organisms appeared in soils supporting the best development of higher plants.

Influence of plant development upon nitrification. The rapidity of accumulation of nitrate nitrogen in soils need not be associated with the abundance of nitrifying organisms. Even though such organisms occur in abundance and even though considerable ammoniacal nitrogen be added, nitrate nitrogen may not accumulate for a considerable period of time in case any abundance of decomposable non-nitrogenous organic matter be present.

King and Whitson (22) found that nitrates accumulate in soil more rapidly after growth of clover than corn or oats (see also Löhnis, 28, p. 766–770). Lyon, Bizzell, B. D. Wilson and J. K. Wilson (31, 32, 33, 34, 37, 38, 69, 70) noted that greater amounts of nitrate nitrogen accumulated under legumes than non-legumes and that nitrification subsequent to removal of the crops was greater from the soils which had grown legumes. Brown (5, 6) found that clover in rotations favored nitrification. Greaves (15) however, reported that nitrification in alfalfa soil was lower than that in wheat soil. Although it would appear from Löhnis' results (29) that nitrification proceeded more rapidly under plants than where no plants were growing, laboratory tests failed to indicate that plant development modified the nitrifying capacity of the soils. However, the method used for estimating this reaction could hardly be expected to detect such differences.

It was concluded by Lyon and his co-workers that the striking effect of legumes was associated with the nitrogen content of the root residues from the plants; the non-legumes were believed to introduce into the soil, organic matter of lower nitrogen content than the legumes. Roots added to soils have been shown to depress nitrate formation in direct order of the percentage content of nitrogen in the roots (36). Information from many sources shows that the consumption of nitrogen in the nutrition of soil microörganisms, developing upon non-nitrogenous organic matter, determines the extent of nitrification (12, 48, 54, 65 and 66 chapter 26).

Lyon and Bizzell (33) found that, in the early stages of growth, plants accelerate nitrification but that at the periods of more advanced development they depress nitrification. Cropping, in general, but cropping to legumes in particular favored nitrification. This enhanced ability of the alfalfa soil to form nitrates persisted for more than a year after the crop was removed. Greaves, Stewart and Hirst (16) observed that plant growth favored the nitrification process.

In general, it appears justifiable to consider, from the information available, that development of higher plants results in modifications of the soil popula-

tion and that marked increases in the abundance of certain microörganisms frequently occur. Certain activities associated with these organisms are also consequently affected.

EXPERIMENTAL PROCEDURE

If it were to be expected that development of higher plants would exert effects upon the soil organisms it seemed reasonable to assume that pronounced effect would be most likely to appear when plant development was well advanced. Preliminary observations were consequently made of soils supporting a variety of plants in the fall of the year. The plants used and the condition of the plants at the time of sampling were as follows:

Sugar beets—plants well developed with heavy roots, vigorous vegetative growth, plants in condition to be harvested.
Alfalfa—second cutting of plants six months old, vigorous vegetative growth.
Field corn—plants approaching maturity, kernels still somewhat soft, plants still green.
Eggplant—had been yielding fruit for several weeks, vegetative growth still green.
Rye—fall cover crop about six weeks old, about eight inches high, vigorous vegetative growth.
Apple tree—about ten years old, fruit had already been harvested this season, leaves still green.

Samples of soil were collected from as near to the roots as possible and in some cases, as with the sugar beets, the soil was taken from the roots subsequent to removing the plants from the soil. At the same time, samples of soil were taken about a foot distant from the plants at the same depth as sampled from the roots but taken so as to include as few roots as possible or none at all. The soils were passed through a 3 mm. sieve and thoroughly mixed before studying.

The following biological observations were made upon these soils:

1. Determination of the abundance of colonies of filamentous fungi developing upon Waksman's acid agar (64). In preparing this medium for use, instead of adjusting the reaction of the medium before sterilization, the acid was added just before pouring the plates. Before sterilization, the medium was titrated with 0.2 *N* acid and calculations were made to determine how much acid should be added to 300 cc. of the medium to bring the reaction to pH 3.8. The medium was sterilized in 300 cc. portions in 500 cc. Erlenmeyer flasks. Standard 0.2 *N* H_2SO_4 was sterilized in a separate flask at the same time. At the time of pouring the plates, when the medium was melted the calculated amount of acid was added with a sterile graduated pipette. Adjustment of the reaction by this means insured a firm medium. Such a method of adjusting the reaction would permit lowering the pH below that possible where the acid was added before sterilization.
2. Determination of the abundance of colonies of actinomyces developing upon Waksman's albumin agar (14, medium 5).
3. Determination of the abundance of colonies of bacteria developing upon the albumin agar.
4. Determnation of the abundance of colonies developing upon nitrogen-free mannite agar (14, medium 77).

5. Determination of the abundance of mucoid colonies developing upon the nitrogen-free mannite agar.
6. Determination of the carbon dioxide evolved in fifteen days from portions of soil calculated to be the equivalent of 100 gm. oven-dry soil.

For the plate counts five plates were counted at the dilution at which the soils were plated. Only averages of these counts are reported.

EXPERIMENTAL RESULTS

The results of these observations are presented in table 2 and figure 1. In the figure, the unshaded columns are extended below the zero line to represent the abundance of organisms and evolution of carbon dioxide in the soils obtained from a distance from the plant roots. The black columns represent the results of the measurements made upon the soils obtained from the rhizosphere. These black columns extend as far below the zero line as the unshaded columns. Consequently any extension of the black columns above the zero line represents the detection of greater numbers of organisms or greater evolution of carbon dioxide in the soil near the plant roots than in the soil distant from the roots. The greater the extension of the black columns above the zero line the greater the determined abundance of organisms or evolution of carbon dioxide in the soils about the roots of the plants concerned.

It is quite apparent that all of the groups of organisms studied appeared in greater abundance in the soils close to the plant roots than in similar soils devoid of root development. These differences appear to be of such an order as to be significant in all cases. Differences are evident between the extent of the influences of root development upon the different groups of organisms and also upon the influences of different plants upon any single group of organisms. It is not intended that these results should be accepted as reflecting the influences which these various plants may exert upon the soil population at any period during the growing season. On the other hand, it is very apparent from the results which will appear in subsequent papers that the plants exert very different effects upon the soil population at different stages in their development. These data however, would seem to indicate that, at any one time, particularly when the plants are well advanced in growth, the microörganisms are more numerous in the soil which has been penetrated by the plant roots.

Of all the organisms studied, the fungi appeared to be least affected by root development. The increases varied between 6 per cent and 80 per cent. The average increase in fungi under all of the plants was 39 per cent. Although these differences may be considered to be significant, the determination of fungi by the plate method is not sufficiently accurate to permit the assumption that slight differences are more than suggestive.

The actinomyces appear to be affected to a somewhat greater extent than the fungi. The increases due to development of plants varied between 23 per cent and 275 per cent. The average increase under all plants was 115

BLE

Influence of plant development upon microörganisms in the soil

PLANT	REGION OF SAMPLING	FILAMENTOUS FUNGI		ACTINOMYCES		BACTERIA		TOTAL COLONIES ON MANNITE		MYCOID COLONIES ON MANNITE		CO_2 EVOLVED IN 15 DAYS	
		Numbers	Increase due to plants	Numbers	Increase due to plants	Numbers	Increase due to plants	Numbers	Increase due to plants	Numbers	Increase due to plants	Amounts	Increase due to plants
			per cent	*millions*	*per cent*	*millions*	*per cent*	*millions*	*per cent*	×100,000	*per cent*	*mgm.*	*per cent*
Apple tree	Near roots	88,000	57	3.2	129	17.4	195	17.6	329	22.8	2,750	11.0	214
	Away from roots	56,000	..	1.4	...	5.9	...	4.1	...	0.8	.....	3.5	...
Rye	Near roots	216,000	33	4.4	38	28.6	117	16.0	191	22.4	300	8.9	19
	Away from roots	162,000	..	3.2	...	13.2	...	5.5	...	5.6	.....	7.5	...
Corn	Near roots	178,000	33	13.4	52	41.0	69	35.8	92	23.4	117	18.2	52
	Away from roots	134,000	..	8.8	...	24.3	...	18.6	...	10.8	.....	12.0	...
Sugar beet	Near roots	222,000	26	15.0	23	57.8	80	53.8	63	29.8	176	17.5	84
	Away from roots	176,000	..	12.2	...	32.1	...	33.0	...	10.8	.....	9.5	...
Egg plant	Near roots	144,000	80	9.0	275	85.0	445	47.6	198	27.0	34	12.5	112
	Away from roots	80,000	..	2.4	...	15.6	...	16.0	...	20.2	.....	5.9	...
Alfalfa	Near roots	268,000	6	9.0	173	93.8	427	63.0	189	116.0	616	20.4	146
	Away from roots	254,000	..	3.3	...	17.8	...	21.8	...	16.2	.....	8.3	...
Average increase due to plants (per cent)			39	....	115	....	222	....	177	.....	666	....	105

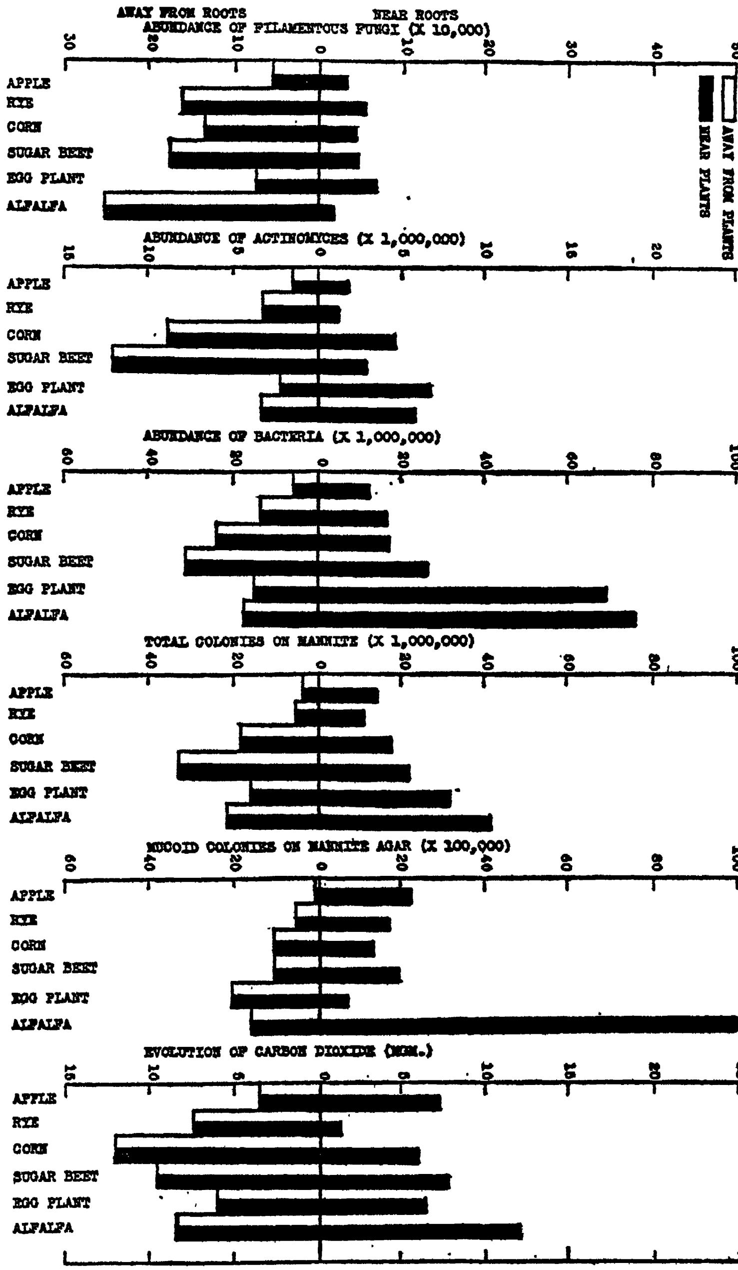


FIG. 1. INFLUENCE OF DEVELOPMENT OF PLANT ROOTS UPON THE ABUNDANCE OF MICROORGANISMS IN SOIL AND THE FORMATION OF CARBON DIOXIDE

per cent. The plate method of determining the abundance of actinomyces in soils may be considered as reflecting the abundance of these organisms in the soil more accurately than such a measurement of fungi.

The numbers of bacteria developing upon albumin agar are affected to a striking degree by plant development. The increases in numbers varied between 69 per cent and 445 per cent. The average of the increases under all plants was 222 per cent. The total colonies developing upon nitrogen-free mannite agar represent much the same organisms as occur upon albumin agar and do not represent nitrogen-fixing bacteria. The effects of plant development upon the abundance of these organisms is much the same as the effects upon the bacteria developing upon albumen agar.

The mucoid colonies developing upon the nitrogen-free mannite agar include such organisms as *B. radiobacter*, *Aero. aerogenes*, *B. radicicola* and related forms which are non-spore-forming, gram-negative rods which may or may not fix nitrogen. This group was increased to a greater degree by root development than any of the other groups of organisms studied. Although the percentage increase was greatest about the roots of the apple tree, by far the greatest increase in numbers of the organisms appeared about the roots of alfalfa.

The only measurement made of the biological activity in these soils was the evolution of carbon dioxide. This is a more exact measurement of the biological condition of soils than the enumeration of any of the organisms by plate counts, consequently, slight differences in formation of the gas may be considered to be significant where similar differences in plate counts would seem to be within the error of the determination. In all cases considerably greater amounts of carbon dioxide were formed by soils obtained from plant roots than by soils supporting no root growth. The average increase due to plant growth was 105 per cent.

It is suggestive that the rye exerted the least influence upon formation of carbon dioxide of all the plants studied, and that the apple roots exerted the greatest proportional increase. This may appear more suggestive in view of the fact that the rye plants were very young while the roots of the apple tree had been developing in the soil over an extended period of years.

These data as a whole suggest that higher plants may affect certain groups of organisms to a different degree than others and that the order of the extent of the influence of growth of different plants upon different groups of organisms may not be the same.

SUMMARY

Material is presented introductory to a series of observations upon the influences of development of higher plants upon the microbial population of soils. The preliminary experimental observations indicate that microörganisms occur in greater abundance about plant roots than at a distance from the roots. Such appeared to be the case with all of the organisms studied:

filamentous fungi, actinomyces, bacteria developing upon albumin agar, organisms developing upon nitrogen-free mannite agar, and, the group of bacteria related to *B. radiobacter* which develop as mucoid colonies upon nitrogen-free mannite agar. Different plants affected any one group of microörganisms differently and caused greater proportional and absolute increases in the abundance of certain groups of organisms than others. Organisms related to the *B. radiobacter* group, bacteria developing upon albumin agar and organisms developing upon nitrogen-free mannite agar increased to a greater degree as a result of plant growth than did the actinomyces or filamentous fungi.

Soils supporting root development produced much more carbon dioxide than soils devoid of roots.

REFERENCES

(1) Aberson, J. H. 1910 Ein Beitrag zur Kenntnis der Natur der Wurzelausscheidungen. *Jahr. f. Wiss. Botan.*, v. 47: 41–56.

(2) Barakov, P. 1910 The carbon dioxide of soils during different stages of growth of plants. *Zhur. Opiutn. Agron.* (Russian *Jour. Exper. Landw.*), v. 11: 321–343. (See *Exp. Sta. Rec.*, v. 23: 523.)

(3) Batchelor, H. W., and Curie, I. H. 1929 A practical method for determining the number of *Clostridium butyricum* colonies in acid soil. *Jour. Bact.*, v. 17: 25–26.

(4) Beijerinck, M. W. 1908 Fixation of free atmospheric nitrogen by Azotobacter in pure culture. Proc. of the Section of Sciences, Kon. Akad. v. Wetenschappen, Amsterdam, v. 11: 67–74. Also in Versamelde Geschriften van M. W. Beijerinck, v. 4: 1921, p. 298–304.

(5) Brown, P. E. 1912 Bacteriological studies of field soils. II. The effects of continuous cropping and various rotations. Iowa Agr. Exper. Sta., Res. Bul. 6: 214–246.

(6) Brown, P. E. 1915 Bacterial activities and crop production. Iowa Agr. Exper. Sta., Res. Bul. 25: 359–388.

(7) Cannon, W. A. 1925 Physiological features of roots with especial reference to the relation of roots to aeration of the soil. With a chapter by E. E. Free on differences between nitrogen and helium as inert gases in anaerobic experiments on plants. Carnegie Inst. of Wash., Pub. 368: 1–168.

(8) Clements, F. E. 1921 Aeration and air-content. The rôle of oxygen in root activity. Carnegie Inst. of Wash., Pub. 315: 1–183.

(9) Cranner, B. H. 1922 Zur Biochemie und Physiologie der Grenzschichten lebender Pflanzenzellen. *Meld. fr. Norges Landbruckshoiskole*, v. 1–2: 1–160.

(10) Creuzburg, U. 1928 Untersuchungen über den Einfluss der Pflanzenbestandes auf das Bakterienleben im Boden. *Landw. Jahr.*, v. 68: 75–115.

(11) Czapek, F. 1896 Zur Lehre von den Wurzelausschiedungen. *Jahr. f. Wiss. Botan.*, v. 29: 321–390.

(12) Doryland, C. J. T. 1916 The influence of energy material upon the relation of soil microörganisms to soluble plant food. No. Dak. Agr. Exp. Sta., Bul. 116: 319–401.

(13) Fred, E. B., and Haas, A. R. C. 1918–19 The etching of marble by roots in the presence and absence of bacteria. *Jour. Gen. Physiol.*, v. 1: 631–638.

(14) Fred, E. B., and Waksman, S. A. 1928 Laboratory manual of general microbiology. McGraw-Hill Book Co., Inc., New York, p. 1–145.

(15) GREAVES, J. E. 1914 A study of the bacterial activities of virgin and cultivated soils. *Cent. f. Bakt.* etc., Abt. II, v. 41: 444–459.

(16) GREAVES, J. E., STEWART, R., AND HIRST, C. F. 1917 Influence of crop, season and water on the bacterial activities of the soil. *Jour. Agr. Res.*, v. 9: 293–341.

(17) HEADDEN, W. P. 1927 The effects of clover and alfalfa in rotation. Colo. Agr. Exper. Sta., Bul. 319: 1–71.

(18) HOFFMAN, C. 1914–15 A contribution to the subject of the factors concerned in soil productivity. Kans. Univ. Sci. Bul. 9: 81–99.

(19) JOHNSON, B. R., AND LEVINE, MAX 1917 Characteristics of coli-like microörganisms from the soil. *Jour. Bact.*, v. 2: 379–401.

(20) JONES, F. R. 1924 A mycorrhizal fungus in the roots of legumes and some other plants. *Jour. Agr. Res.*, v. 29: 459–470.

(21) JOSHI, N. V. 1925 Studies on the root nodule organism of the leguminous plants. Agr. Res. Inst. (Pusa) India, Mem. of Dept. of Agr., *Bact. Ser.*, v. 1: 247–276.

(22) KING, F. H., AND WHITSON, A. R. 1900 Soluble salts of cultivated soils. Wis. Agr. Exper. Sta., 17th Ann. Rpt., p. 204–226.

(23) KLEIN, M. A. 1915 Studies in the drying of soils. *Jour. Amer. Soc. Agron.*, v. 7: 49–77.

(24) KNOP, W. 1864 Chemische Untersuchungen über die Ernährung der Pflanze. *Ann. Chem. Pharm.*, v. 129: 298–330.

(25) KUNZE, G. 1906 Über Säureausscheidungen bei Wurzeln und Pilzhyphen und ihre Bedeutung. *Jahr. f. Wiss. Botan.*, v. 42: 357–393.

(26) LEBEDJANTZEV, A. N. 1924 Drying of soil as one of the natural factors in maintaining soil fertility. *Soil Sci.*, v. 18: 419–447.

(27) LECLAIR, C. A. 1915 Influence of growth of cowpeas upon some physical, chemical and biological properties of soils. *Jour. Agr. Res.*, v. 5: 439–448.

(28) LÖHNIS, F. 1910 Handbuch der landwirtschaftlichen Bakteriologie. Geb. Borntraeger, Berlin, p. 1–905.

(29) LOHNIS, F. 1926 Effect of growing legumes upon succeeding crops. *Soil Sci.*, v. 22: 355–389.

(30) LUNDEGARDH, H. 1924 Der Kreislauf der Kohlensäure in der Natur. Jena, Gustav Fischer, p. 1–308.

(31) LYON, T. L., AND BIZZELL, J. A. 1911 A heretofore unnoted benefit from the growth of legumes. N. Y. (Cornell) Agr. Exper. Sta., Bul. 294: 365–374.

(32) LYON, T. L., AND BIZZELL, J. A. 1913 The influence of alfalfa and timothy on the production of nitrates in soils. *Cent. f. Bakt.* etc., Abt. II, v. 37: 161–167.

(33) LYON, T. L., AND BIZZELL, J. A. 1913 Some relations of certain higher plants to the formation of nitrates in soils. N. Y. (Cornell) Agr. Exper. Sta., Mem. 1: 1–111.

(34) LYON, T. L., BIZZELL, J. A., AND WILSON, B. D. 1920 The formation of nitrates in a soil following the growth of red clover and of timothy. *Soil Sci.*, v. 9: 53–64.

(35) LYON, T. L., AND WILSON, J. K. 1921 Liberation of organic matter by roots of growing plants. N. Y. (Cornell) Agr. Exper. Sta., Mem. 40: 1–44.

(36) LYON, T. L., BIZZELL, J. A., AND WILSON, B. D. 1923 Depressive influence of certain higher plants on the accumulation of nitrates in soil. *Jour. Amer. Soc. Agron.*, v. 15: 457–467.

(37) LYON, T. L., BIZZELL, J. A., AND WILSON, B. D. 1924 An inquiry into the reason for the large accumulation of nitrates in soil following the growth of clover or alfalfa. *Jour. Amer. Soc. Agron.*, v. 16: 396–405.

(38) LYON, T. L., AND WILSON, B. D. 1928 Some relations of green manures to the nitrogen of a soil. N. Y. (Cornell) Agr. Exper. Sta., Mem. 115: 1–29.

(39) MELIN, E. 1924 Die Phosphatide als ökologischer Faktor im Boden. *Svensk. Bot. Tidskr.*, 18.

(40) MELIN, E. 1925 Untersuchungen über die Bedeutung der Baummykorrhiza. Jena, Gustav Fischer, p. 1–152.
(41) METZGER, W. H. 1928 The effect of growing plants on solubility of soil nutrients. *Soil Sci.*, v. 25: 273–280.
(42) NELLER, J. R. 1922 The influence of growing plants upon oxidation processes in the soil. *Soil Sci.*, v. 13: 139–160.
(43) NEWTON, J. D. 1923 A comparison of the absorption of inorganic elements and of the buffer systems of legumes and non-legumes and its bearing upon existing theories. *Soil Sci.*, v. 15: 181–204.
(44) NEWTON, J. D. 1924 Measurements of carbon dioxide evolved from the roots of various crop plants. *Scientific Agriculture*, v. 4: 268–274.
(45) PARKER, F. W. 1924 Carbon dioxide production of plant roots as a factor in the feeding power of plants. *Soil Sci.*, v. 17: 229–247.
(46) PARKER, F. W. 1927 Soil phosphorus studies. III. Plant growth and the absorption of phosphorus from culture solutions of different phosphorus concentrations. *Soil Sci.*, v. 24: 129–146.
(47) PEROTTI, R. 1926 On the limits of biological inquiry in soil science. Proc. Inter. Soc. *Soil Sci.*, v. 2: 146–161.
(48) RAHN, O. 1919 Die schädliche Wirkung der Ströhdungung und deren Verhütung. *Zeit. f. tech. Biol.*, v. 7: 172–186.
(49) RAYNER, M. C. 1927 Mycorrhiza. New Phytologist Reprint, No. 15. Wheldon and Wesley, Ltd., p. 1–246.
(50) RIKER, A. J., BANFIELD, W. M., AND WRIGHT, W. H. 1928 The relation of certain bacteria to the development of roots. *Science*, n.s., v. 68: 357–359.
(51) ROKITSKAIA, A. I. 1926 Aerobic fermentation of cellulose under the influence of the soil microflora in the zone of the plant root system. Izv. Nauch. Melior. Inst., No. 13: 168–208.
(52) SACHS, J. 1860 Physiologische Mittheilungen verschiedenen Inhaltes: II Auflösung des Marmors durch Maizwurzeln. *Bot. Zeit.*, v. 18: 117–119.
(53) SMITH, N. R. 1928 The identification of B. radiobacter and its occurrence in soil. *Jour. Bact.*, v. 15: 20–21.
(54) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.*, v. 17: 293–314.
(55) STOKLASA, J. 1924 Über die Resorption der Ionen durch das Wurzelsystem der Pflanzen aus dem Boden. *Ber. d. Deut. Botan. Gesell.*, v. 42: 183–191.
(56) STOKLASA, J. 1924 Die modernen Zeile der biochemischen Forschung des Bodens. *Chemie d. Zelle u. Gewebe*, v. 12: 23–44.
(57) STOKLASA, J., AND DOERELL, E. G. 1926 Handbuch der biophysikalischen und biochemischen Durchforschung des Bodens. Paul Parey, Berlin, p. 1–812.
(58) STOKLASA, J., AND ERNEST, A. 1905 Ueber den Ursprung, die Menge und die Bedeutung des Kohlendioxyds im Boden. *Cent. f. Bakt.* etc., Abt. II, v. 14: 723–736.
(59) STOKLASA, J., AND ERNEST, A. 1909 Beiträge zur Lösung der Frage der chemischen Natur des Wurzelsekretes. *Jahr. f. Wiss. Botan.*, v. 46: 55–102.
(60) TARANOVSKAIA, V. G., AND GERMANOV, F. N. 1927 Nitrification in the soil of the experimental fields of the Nosovka Agricultural Experimental Station and the rôle of clover in its improvement. Nosovsk. Selsk. Khoz. Opytn. Sta., Bul. 45: 1–48. (See *Exper. Sta. Rec.*, v. 59: 117.)
(61) TRUFFAUT, G., AND BEZSSONOV, N. 1925 Fixation de l'azote gaseux par les plantes supérieures autres que les legumineuses. *Sc. Sol.*, v. 4: 3–53.
(62) TURPIN, H. W. 1920 The carbon dioxide of the soil air. N. Y. (Cornell) Agr. Exper. Sta., Mem. 32: 319–362.

(63) Velich, A. 1903 Bakteriologische Untersuchung der Zuckerrübenwurzelfasern. *Zeit. f. Zuckerindustrie in Böhnen*, v. 27: 975. (See *Cent. f. Bakt.*, etc. Abt. II, v. 10: 678–679.)
(64) Waksman, S. A. 1922 A method for counting the number of fungi in the soil. *Jour. Bact.*, v. 7: 339–341.
(65) Waksman, S. A. 1924 Influence of microörganisms upon the carbon-nitrogen ratio in the soil. *Jour. Agr. Sci.*, v. 14: 555–562.
(66) Waksman, S. A. 1927 Principles of soil microbiology. The Williams & Wilkins Co., Baltimore, p. 1–897.
(67) Waksman, S. A., and Starkey, R. L. 1923 Partial sterilization of soil, microbiological activities and soil fertility: II. *Soil Sci.*, v. 16: 247–268.
(68) Wilson, J. K., and Lyon, T. L. 1926 The growth of certain microörganisms in planted and unplanted soil. N. Y. (Cornell) Agr. Exper. Sta., Mem. 103: 1–25.
(69) Wilson, B. D., and Wilson, J. K. 1925 An explanation for the relative effects of timothy and clover residues in the soil on nitrate depression. N. Y. (Cornell) Agr. Exp. Sta., Mem. 95: 1–21.
(70) Wilson, B. D., and Wilson, J. K. 1928 Relation of sorghum roots to certain biological processes. *Jour. Amer. Soc. Agron.*, v. 20: 747–754.
(71) Wilfarth, H., Römer, H., and Wimmer, G. 1906 Über die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. *Land. Vers.-Stat.*, v. 63: 1–70.

Volume XXVII May, 1929 Number 5

SOIL SCIENCE

Contents

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
New Brunswick, N. J.

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to Dr. Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

SOIL SCIENCE is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Volumes begin with the January and July issues, respectively. The subscription price is $10.00 for Volumes XXV (Nos. 1–6 inclusive) and XXVI (Nos. 1–6 inclusive) January, 1928, to December, 1928, inclusive, United States, Mexico, Cuba. An additional charge per volume of 50 cents for subscriptions in other countries. *Back volumes* are supplied on orders for Vols. I to XIV inclusive, XIX to XXIV incl.—$100.00 per set.

New subscriptions are accepted to begin with the current issue, unless the subscriber gives instructions to begin the subscription with some other issue. If the subscriber desire, back issues of the current volume may be obtained when in stock. The publishers do not guarantee to supply back issues, however.

Renewal subscriptions. To avoid a break in your series, subscriptions should be renewed promptly on expiration. Otherwise it may prove impossible for the publishers to furnish the back issues missed, as only sufficient copies are printed of each issue to supply orders in hand.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; or G. Jervis Manton, 430 Lit. Collins St., Melbourne (and at Sydney and Brisbane)

For the British Empire, except Australia and Canada: Bailliere, Tindall & Cox; 8 Henrietta Street, Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann, Krausenstrasse 38, Berlin N. W., Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana. 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE. U. S. A.

Publishers of: *Medicine, Journal of Urology, Bulletin of the Johns Hopkins Hospital, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Physical Therapeutics, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thompson Research Laboratory, Journal of Biological Chemistry, Chemical Reviews. Journal of Dairy Science, Soil Science. Social Forces, Personnel Journal, Journal of Comparative Psychology. Comparative Psychology Monographs, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, American Speech, Childhood Education.*

DETECTION AND SIGNIFICANCE OF MANGANESE DIOXIDE IN THE SOIL

W. O. ROBINSON

Bureau of Chemistry and Soils, United States Department of Agriculture

Received for publication January 4, 1929

Work on the determination of organic matter in soils by means of hydrogen peroxide revealed that a sample of Blakely loam decomposed the hydrogen peroxide so rapidly that the organic matter was not destroyed. The soil contained an unusual quantity of manganese and had a peculiar, dark brown color. Preliminary tests indicated that the Blakely loam and certain lateritic soils having a similar color, high manganese content, and high hydrogen peroxide activity contained manganese in the dioxide form.

This study was undertaken to determine whether the hydrogen peroxide activity of a soil could be taken as a positive indication of the presence of manganese dioxide, and if so, to study the distribution of manganese dioxide in some of the soil series.

PREVIOUS WORK ON THE OCCURRENCE OF MANGANESE DIOXIDE IN SOILS

Concretions of manganese dioxide have been noted in the soil by Doherty (6), Thresh (28), and Helbig (11). Worsham et al. (31) have reported the presence of manganese dioxide in the dark colored Davidson Clay in Georgia. Manganese dioxide in large quantities has been reported in Hawaiian soils by Kelly (13). McGeorge (15) and Johnson (12) have reported further occurrences of manganese dioxide in Hawaiian soils. Johnson gives some quantitative data based on the determination of the oxygen evolved on heating the soil. Bertrand (2) makes the general statement that manganese occurs in the soil in several different forms, including the dioxide. Sharrer (25) has shown that there is a correlation between the catalytic power of a soil and the manganese and iron content.

HYDROGEN PEROXIDE TEST FOR MANGANESE DIOXIDE IN THE SOIL

Platinum black, metallic peroxides, charcoal, chromium trioxide, and manganese dioxide decompose hydrogen peroxide with great energy. Of all these substances, manganese dioxide is the only one likely to be of any considerable occurrence in soils.[1]

[1] It has been stated that ferric oxide will decompose hydrogen peroxide rapidly. Much depends upon the samples of ferric oxide used. Many red soils containing as high as 34 per cent Fe_2O_3 will not decompose hydrogen peroxide rapidly.

It has been stated also that charcoal is a common constituent of soils. It is only rarely, however, that the quantities involved are sufficient to cause rapid decomposition of hydrogen

TABLE 1

Relation between total manganese and capacity of the soil material to decompose hydrogen peroxide

SOIL	DEPTH	TOTAL MANGANESE AS MnO	RATE OF DECOMPOSITION OF H_2O_2
	inches	*per cent*	
Blakely loam, Georgia*	0–15	0.50	200 cc. in 6 minutes
	15–24	0.22	200 cc. in 20 minutes
	24–48	0.10	30 cc. in 30 minutes
	48–60	0.03	No activity
	60–96	0.37	200 cc. in 18 minutes
	96–116	0.38	200 cc. in 12 minutes
Blakely clay loam, Georgia	0–3	0.68	200 cc. in 9 minutes
	3–10	0.84	200 cc. in 7.9 minutes
	10–22	0.52	185 cc. in 30 minutes
	22–45	0.19	Very little activity
	45–70	0.17	Practically no activity
Blakely clay loam, Georgia	0–1½	0.53	200 cc. in 10 minutes
	1½–7	0.49	200 cc. in 17 minutes
	7–20	0.33	200 cc. in 24 minutes
	20–45	0.15	30 cc. in 30 minutes
	45–60	0.53	200 cc. in 11 minutes
Blakely clay loam, Georgia	0–½	1.20	200 cc. in 2.9 minutes
	½–3	1.27	200 cc. in 2.5 minutes
	3–15	0.89	200 cc. in 3.3 minutes
	15–36	0.50	200 cc. in 28.0 minutes
Decatur silt loam, Ala*	0–3	0.23	95 cc. in 30 minutes
	3–6	0.28	88 cc. in 30 minutes
	6–10	0.15	82 cc. in 30 minutes
	10–18	0.03	5 cc. in 30 minutes
	18–40	0.03	7 cc. in 30 minutes
	40–70	0.02	4 cc. in 30 minutes
Dicklom loam, Philippines	0–6	0.57	130 cc. in 30 minutes
	6–36	0.23	85 cc. in 30 minutes
	36–48	0.18	55 cc. in 30 minutes
Greenville sandy loam, Alabama*	0–4	0.17	20 cc. in 30 minutes
	4–48	0.04	No activity
	48–78	2.85	200 cc. in 1.7 minutes
	78–114	0.03	No activity
Greenville sandy loam, Georgia	0–2	0.09	Practically no activity
	2–9	0.05	Practically no activity
	9–40	0.02	Practically no activity
	40–52	0.03	Practically no activity
	52–55	1.02	200 cc. in 21 minutes
	55–70	0.02	No activity

* Analysis for MnO by G. Edgington, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

TABLE 1—*Continued*

SOIL	DEPTH	TOTAL MANGANESE AS MnO	RATE OF DECOMPOSITION OF H_2O_2
	inches	*per cent*	
Hagerstown loam, Tennessee	0–4½	0.44	137 cc. in 30 minutes
	4½–11	0.37	110 cc. in 30 minutes
	11–24	0.27	80 cc. in 30 minutes
	24–36	0.34	50 cc. in 50 minutes
Lurugan loam, Phillippines	0–8	0.80	200 cc. in 11 minutes
	8–36	0.44	170 cc. in 30 minutes
	36–48	0.43	Little activity
Marshall silt loam, (Phase of) Kansas	0–13	0.12	30 cc. in 30 minutes
	13–25	0.13	35 cc. in 30 minutes
	25–32	0.16	63 cc. in 30 minutes
	32–54	0.19	55 cc. in 30 minutes
	54–62	0.11	33 cc. in 30 minutes
	62+	1.03	200 cc. in 0.5 minute
Summit silt loam, Kansas	0–8	0.07	15 cc. in 30 minutes
	8–14	0.12	25 cc. in 30 minutes
	14–26	0.27	50 cc. in 30 minutes
	26–48	0.32	200 cc. in 11 minutes
	48–72	0.19	115 cc. in 30 minutes

The power of a number of soils to decompose hydrogen peroxide and the manganese content of these soils have been compared. For this purpose profile samples of the soil were taken. One gram of the soil was put in a flask connected with a gas burette, 10 cc. H_2O and 10 cc. 30 per cent H_2O_2 were introduced, and the gas evolved in 30 minutes, or the time necessary to evolve 200 cc. was measured. The flask was shaken every minute. The peroxide used was Merck's "Superoxol." It was only very slightly acid and contained some preservative. It was not made neutral or slightly alkaline with NaOH as is done in soil studies where the activity of the soil catalase is involved.

The results of the test, given in table 1, show that high manganese content of the soil is associated with high hydrogen peroxide activity.

The question arises as to what combination of manganese is responsible for the very rapid decomposition of the peroxide. From the sandy fractions of all the samples given in table 1 which showed a hydrogen peroxide activity of 200 cc. in less than 30 minutes, a number of black or dark brown concretions could be separated. These concretions would decompose hydrogen peroxide

peroxide. Of about fifty soils which contained charcoal, only one contained enough to decompose hydrogen peroxide rapidly. This soil was from the "charcoal plot" at the Pennsylvania experiment station. It contained about 5 per cent charcoal and was once the site of a charcoal furnace.

vigorously, particularly when finely ground. Further, if small quantities of the sandy fractions were scattered loosely on the bottom of a beaker and covered with dilute H_2O_2, the origin of the arising bubbles could invariably be traced to the black concretions. Samples of hand picked concretions yielded on analysis from 8.28 per cent MnO_2 in the Scott silt loam to 34 per cent in the Blakely loam. These concretions gave off oxygen when heated and caused chlorine to be given off from hydrochloric acid. There can be no doubt that the concretions were impure manganese dioxide. For the sake of brevity, these concretions will be called "manganese concretions." They commonly contain much more ferric oxide and other soil material than they do manganese dioxide.

TABLE 2

Relation of total manganese in soil fractions to the power to decompose hydrogen peroxide

SOIL AND FRACTION	MnO	RATE OF DECOMPOSITION
	per cent	
Blakely loam, 16–24 inches:		
Sand	0.21	100 cc. in 30 minutes
Silt	1.23	200 cc. in 1.5 minutes
Colloid	0.54	23 cc. in 30 minutes
Greenville sandy loam, 48–78 inches:		
Sand	1.87	200 cc. in 1.4 minutes
Silt	4.93	200 cc. in 0.1 minute
Colloid	1.02	38 cc. in 30 minutes
Orangeburg fine sandy loam, 10–36 inches:		
Sand	0.18	Very little activity
Silt	0.67	55 cc. in 30 minutes
Colloid	0.59	30 cc. in 30 minutes
Clarksville silt loam:		
Colloid	0.37	30 cc. in 30 minutes
Manor loam:		
Colloid	0.11	7 cc. in 30 minutes
Susquehanna clay:		
Colloid	0.30	5 cc. in 30 minutes
Vega Baja clay loam:		
Colloid	0.16	15 cc. in 30 minutes

W. H. Fry, of this Bureau, reported that the sands and silts from the Blakely and Greenville surface soils contained no silicates or carbonates of manganese that could be discovered petrographically by using a reasonable quantity of sample. There is very little probability of manganese silicates or carbonates being present in such highly weathered soils as the Blakely and Greenville. Finely ground manganese silicates do not decompose hydrogen peroxide.

It seems pretty well established that manganese concretions were responsible for the hydrogen peroxide activity of the sandy fractions of the samples given in table 1. Further light might be shed on the problem by studying the

manganese content and hydrogen peroxide activity of the sand, silt, and clay fractions. These separations were made in the case of three soils. The data are given in table 2, together with the data of a number of soil colloids containing considerable manganese.

The data given in table 2 show that the silt fractions were the highest in manganese and the most active in decomposing H_2O_2. This is in harmony with the studies of McGeorge (15), who found that the silt and very fine sand fractions of a number of Hawaiian soils contained the most manganese and that the manganese was largely in the form of manganese dioxide.

It is surprising that the clay, having a relatively enormous surface, and a comparatively high manganese content, should be so inactive in decomposing hydrogen peroxide. The finer manganese dioxide is ground the more readily will it decompose hydrogen peroxide. Very small quantities of finely ground manganese dioxide when added to the inactive colloids will make them very active in decomposing hydrogen peroxide. We must conclude that the soil colloids examined did not contain manganese dioxide.

TABLE 3

Exchangeable manganese in fractions of Blakely loam and Greenville sandy loam

SOIL	DEPTH	FRACTION	EXCHANGEABLE MANGANESE AS MnO	TOTAL MANGANESE AS MnO
	inches		*per cent*	*per cent*
Blakely loam	16–24	Sand	0.046	0.21
		Silt	0.069	1.23
		Colloid	0.410	0.54
Greenville sandy loam	48–78	Sand	0.042	1.87
		Silt	0.075	4.93
		Colloid	0.330	1.03

For the purpose of throwing some light on the question of the forms of manganese present in the different mechanical soil fractions, the sand, silt, and clay fractions of the Blakely and Greenville soils were tested for exchangeable manganese. One gram samples were washed on a filter paper with 250 cc. of normal ammonium chloride. The manganese was determined in the filtrate. The results of the study are given in table 3.

The data given in table 3 show that a considerable part of the manganese in the colloidal matter is exchangeable while comparatively little of the manganese in the silts and sands is exchangeable. A large part of the manganese in the sands and silts seems to be in the form of the dioxide. Since fine grinding of manganese dioxide increases its activity, and the clay which is very finely divided, contains relatively large quantities of manganese, it is plain there can be little manganese dioxide in the clay. The fact that the manganese in the clay is partly exchangeable and not in the form of manganese dioxide would lead to the belief that the manganese in this fraction occurs as a part of the basic radical of the absorption complex.

QUANTITATIVE ASPECT OF THE HYDROGEN PEROXIDE TEST FOR MANGANESE DIOXIDE

Positive identification of manganese dioxide in the soil is very desirable since the occurrence of manganese dioxide seems to be characteristic of certain series of soil. The determination should be quantitative, if possible, for there is presumably some relation between the quantity of manganese dioxide and certain properties of the soil.

The quantitative methods for the determination of manganese dioxide in ores fail, in general, in the case of soils. This is due to the almost universal presence of organic matter, ferrous iron minerals, and, occasionally, calcium carbonate. The Bunsen method, in which the manganese is estimated by measuring the quantity of chlorine liberated by boiling with hydrochloric acid, would be accurate were it not for the interference of ferrous iron minerals and organic matter and also the apparent very slow liberation of chlorine from the boiling soil solution when no manganese dioxide is present. The evolution of a considerable quantity of chlorine at the first boiling of the soil solution is a positive indication of manganese dioxide.

Brewer and Carr (3) report some quantitative determinations of manganese dioxide in the soil. They modified the Bunsen method (26) by adding mercuric oxide to prevent the chlorination of the organic matter. Their results on synthetic mixtures of soils and considerable quantities of manganese dioxide showed that under these conditions nearly all the manganese added could be detected. The results in the case of two soils were 0.16 per cent MnO_2 too low.

Brewer and Carr recommend a method employing an acid solution of ferrous ammonium sulfate to dissolve the manganese dioxide in the soil. They recognize two forms of manganese, the easily soluble manganese and manganese dioxide. The easily soluble manganese is determined by digesting with 1.1 per cent sulfuric acid. The soil is then digested with a mixed solution containing 1.1 per cent sulfuric acid and 5 per cent ferrous ammonium sulfate. The difference between the manganese dissolved by the mixed solution and that dissolved by the 1.1 per cent sulfuric acid is considered to be manganese dioxide.

Brewer and Carr's method apparently fails with some soils, particularly in soil colloids containing a considerable quantity of manganese. In these cases the acid ferrous ammonium sulfate dissolves considerably more manganese than the sulfuric acid solution alone, although manganese dioxide is apparently not present in soil colloids.

The method employed by Johnson (12) is applicable only to soils free from organic matter. In this method the soils are heated to redness, and the oxygen evolved during heating is a measure of the quantity of manganese dioxide present

Work with synthetic mixtures of various soils with manganese dioxide has

shown that none of the proposed methods are reliable for the determination of manganese in the form of dioxide. Ferrous iron minerals and organic matter may mask as much as as 1 per cent of manganese in methods where the chlorine evolved is taken as a measure of manganese dioxide.

Tests in the foregoing synthetic mixtures have shown the hydrogen peroxide test will detect one-tenth per cent of manganese dioxide in soils. With these synthetic mixtures the rate at which the peroxide is decomposed is proportional to the quantity of manganese dioxide present. This suggested that the test might be quantitative.

It was first established that the rates of oxygen and heat evolution from a mixture of hydrogen peroxide and a soil containing no manganese dioxide but much organic matter were negligible compared to the rate of decomposition when manganese dioxide was present. This statement seemingly contradicts numerous experiments in the measurement of soil catalysis. The conditions are different. In soil catalysis experiments reported by May and Gile (18) and by Sharrer (25) the hydrogen peroxide is comparatively weak and it is

TABLE 4

Time required to reach a maximum temperature and to liberate 1000 cc. oxygen with varying quantities of psilomelane

QUANTITY OF PSILOMELANE	TIME TO REACH MAXIMUM TEMPERATURE	TIME TO EVOLVE 1000 CC.	RISE OF CALORIMETER CONTENTS
gm.	*minutes*	*minutes*	*°C.*
0.10	230	62	27.7
0.15	97	40	28.0
0.25	45	21	28.0

made just barely alkaline with sodium hydroxide. The hydrogen peroxide from such a solution is rapidly decomposed by most highly organic soils and particularly by partially decayed banana peels. Under these conditions the decomposition is rapid in soils containing no manganese dioxide. Loew (14) has shown, however, that when strong hydrogen peroxide such as Merck's "Superoxol" is used the catalase is apparently destroyed. "Superoxol," diluted with its own volume of water is neutral to litmus paper. The decomposition rate of this diluted "Superoxol" by a very fertile garden soil or by banana peel is negligible compared to the decomposition rate when manganese dioxide is present.

To determine the quantitative possibilities of the hydrogen peroxide test, varying quantities of finely ground psilomelane were mixed with 5 gm. of soil showing no reaction with the peroxide and added to 20 cc. 15 per cent hydrogen peroxide in an adiabatic calorimeter. The gas and heat given off were measured and the time noted necessary to reach 1000 cc. gas and the maximum temperature. The data are given in table 4.

The data of table 4 show that the rates of heat and oxygen evolution are

dependent upon the quantities of manganese dioxide present. They also show that the measure of the rate of oxygen evolution is more practical than that of heat evolution.

If the same conditions were present in soils, particularly as to the extent of subdivision of the manganese dioxide, as were present in the experiment just described, one would expect that the method might be used to determine the quantity of manganese dioxide in the soil. The varying surface, however, complicates the case. It has been shown that manganese dioxide occurs in the sands and silts only, and not in the clay. With the elimination of the clay, the chances for great variation in the surface of any particular percentage of manganese dioxide are somewhat restricted. It is hardly to be supposed that there will be an equal size distribution of the manganese dioxide particles in the sands and silts of various soils. If there is not an even size distribution and if the rate at which hydrogen peroxide is decomposed varies very much

TABLE 5

*Relation between size of psilomelane particles and hydrogen peroxide activity**

SIZE OF PSILOMELANE PARTICLES	TIME NECESSARY TO GIVE 1000 CC.	TIME NECESSARY TO REACH MAXIMUM TEMPERATURE
mm. diameter	*minutes*	*minutes*
0.50–0.33	178	218
0.084–0.01	25	4.5
0.01	Too rapid to measure	Too rapid to measure

* For 1 gm. psilomelane. 10 cc. H_2O_2 and 10 cc. H_2O.

with the size of the manganese dioxide particles, then the method can not be used for a quantitative determination of the manganese dioxide present.

Some experiments on the variation of the rate at which hydrogen peroxide is decomposed by various sized particles of manganese dioxide have been made. A sample of psilomelane was ground and sieved into three sizes. The rates at which 1 gm. of each of the sizes decomposed 20 cc. of 15 per cent hydrogen peroxide were measured. The results are given in table 5.

The data given in table 5 show that the state of subdivision has a great deal to do with the rate at which manganese dioxide decomposes hydrogen peroxide. The effect of varying the sizes of the manganese dioxide on the quantitative value of the hydrogen peroxide activity can be seen by assuming that all the manganese dioxide particles in a certain soil A are from 0.50-0.33 mm. diameter and in soil B, from 0.084–.01 mm. diameter. Suppose A and B give identical rates of decomposition of the hydrogen peroxide solution, soil A would have many times more manganese dioxide than soil B. Such differences as the above in size distribution are probably extreme and would not occur in natural soils.

APPROXIMATE METHOD FOR THE DETERMINATION OF MANGANESE DIOXIDE IN SOILS

Johnson (12) has shown that nearly all the manganese in Hawaiian soils high in that element, is in the form of manganese dioxide. Our results show that the weathered manganese is in two forms in soils containing manganese dioxide. A part of it is absorbed by the colloidal complex, the rest is apparently in the form of the dioxide in the sands and silts. Manganese dioxide in soils is a product of rock decay or rock alteration and if the conditions in the soil are such that any manganese dioxide is formed, it is probable that all the manganese in the sands and silts would be in the form of the dioxide. Based on the above assumptions, two methods are suggested for the determination of manganese dioxide in the soil. The hydrogen peroxide test is first used to establish the presence of the dioxide.

The presence of manganese dioxide having been established by the hydrogen peroxide test, the soil may be separated into colloidal and non-colloidal fractions and the total manganese determined in the non-colloidal fraction. According to the assumption all the manganese in the sand and silt is in the form of the dioxide. The chlorine evolved rapidly by the Bunsen method checked fairly well with the total manganese in the sands and silt of the Blakely and Greenville soil materials in which much manganese was present. Another way would be to determine the total manganese and the exchangeable manganese in the whole soil and the difference should represent the maximum quantity of manganese present as manganese dioxide. Not all the manganese in the colloidal matter is exchangeable, it is true, but if the colloidal matter is low and the total manganese is high, the error due to this cause is lessened. Brewer and Carr's (3) method is, however, more advisable when the colloidal matter has not been separated from the non-colloidal matter.

There is little probability of manganese existing as a silicate in the sands and silts of soils containing manganese dioxide. It has been noted that two manganiferous soils did not contain any manganese silicate that could be detected petrographically. In general, any soil containing manganese dioxide is so thoroughly weathered that there is very little likelihood of any undecomposed manganese silicate being present.

In this work it has been shown that manganese dioxide particles could be separated from all soils (about 125 samples) which evolved more than 200 cc. oxygen in 30 minutes from a mixture of 1 gm. of finely ground soil, 10 cc. of "Superoxol," and 10 cc. of water. Occasionally a sample giving only slightly less than 200 cc. has contained a concretion of impure manganese dioxide. On the other hand, an occasional sample has given nearly 200 cc. under like condition, and from these samples no manganese dioxide could be separated. The data shown in table 1 do not indicate a proportionality between the total manganese and the rate of oxygen evolution. Without reasoning in a circle it is impossible to tell how the total manganese is divided between the colloid and

manganese dioxide. However, the rate of oxygen and heat evolution will tell whether there is little, considerable, or a relatively large quantity of manganese present as the dioxide. Although the rate of oxygen evolution of a synthetic mixture of soil and manganese dioxide is dependent on the quantity of manganese dioxide, it has been shown that the rate is dependent upon the extent to which the manganese dioxide is subdivided. The size of the manganese dioxide particles in different soils may not vary greatly, but there is too much uncertainty to assume that they do not. It seems better, therefore, to rely on the hydrogen peroxide test as a qualitative indication of the presence of manganese dioxide and determine the total manganese in the sands and silts or use Brewer and Carr's method, knowing that the quantity of dioxide shown is somewhat greater than that actually present.

SOLUTION OF MANGANESE AND DEPOSITION OF MANGANESE DIOXIDE

Manganese occurs in the lithosphere in very small quantities. Clarke (4) gives the average as 0.11 per cent MnO. The numerical average for soils is about 0.12 per cent, the range being from less than 0.001 per cent to 1.27 per cent in surface soils of the United States. Tropical soils may contain as high as 15 per cent.

Manganese is present in the manganous condition as a minor constituent of igneous rocks. When the rock weathers the manganese is released rather easily, resembling ferrous iron in this respect (19).

Manganese is dissolved and transported as manganous bicarbonate (21) sometimes to a great distance. Chalybeate waters containing both iron and manganese deposit their iron sooner than manganese according to Clarke (4). This property, together with the fact that the manganese content of the lithosphere is small, possibly accounts for the occurrence of relatively few and concentrated deposits of manganese and also for the localized distribution of manganese in the soil profile.

The dissolved manganese in river, spring, and well waters varies greatly. It is generally only a trace but reaches as high as 5 to 10 p.p.m. in carbonated waters (4).

The solubility of manganese carbonate in carbon dioxide waters is given by Gmelin-Kraut (10) as 0.130 gm. per liter and by Ageno and Valla (1) as 0.065 gm. per liter at 25°. According to Roscoe and Schorlemmer (22) manganese carbonate dissolves in water saturated with carbon dioxide to the extent of 0.25 gm. per liter.

Manganese is particularly susceptible to solution and deposition under soil conditions, because of the readiness with which it assumes different states of oxidation and the different solubilities of manganous and manganic compounds. When in the manganous state it is soluble to the extent of several parts per million in meteoric waters. Manganous salts are more stable under atmospheric conditions than are ferrous salts. Manganous manganese exists in the colloidal matter of well-drained surface soils even when the reaction is only slightly acid.

Although manganous solutions are more stable than ferrous iron solutions, they are readily oxidized by various natural agencies to the insoluble dioxide. Murray and Irvine (20) report an example of this oxidation in the Clyde River in Scotland. The headwaters contain manganous bicarbonate in solution. Near the mouth of the river the pebbles near the bank are coated with black manganese dioxide, and suspended particles of manganese dioxide may be detected in the water. In municipal water supply systems manganese bearing waters frequently give trouble by clogging the water mains with precipitated manganese dioxide.

Manganese dioxide is practically insoluble in water. Under certain soil conditions it is very stable and the composition of some lateritic soils would lead to the belief that manganese dioxide is less soluble than iron and aluminum oxides. Although manganese is present in the parent rock in very small quantities and iron and aluminum are present in very large quantities, the manganese may make up several per cent of the lateritic soil.

The solibiluty of manganese in the soil is greatly dependent upon the reaction of the soil and upon whether oxidizing or reducing conditions prevail. Ruprecht and Morse (23) have shown that manganese is one of the easily replaceable bases, and Mattson (17) has shown that it is replaced most easily when the reaction is decidedly acid. This is in line with field experience. Ruprecht and Morse (23) found that the manganese was very soluble in certain Massachusetts plots made acid by the long continued application of ammonium sulfate. Kelly and McGeorge (13) show that the manganese is made more soluble by heating the soil, particularly when the soil is heated high enough to reduce the manganese by the organic matter. In this laboratory it has been found that manganese becomes very soluble in water-logged soils. The solubility of the manganese in a number of air-dried soils from nearby Maryland and Virginia as determined by shaking the air-dried soil with water and letting settle over night, was less than one part per million. When the soil was submerged in water in a stoppered bottle for several days the soluble manganese rose as high as 500 p.p.m. calculated on the weight of the soil.

The conditions which bring about the precipitation of manganese dioxide, though not well known, must be very sharply defined, because very pure deposits are found in media containing only traces of manganese. Large quantities of manganese nodules are found on the surface of certain deep sea deposits. In some soil profiles it is conspicuously absent from some layers but occurs in other layers in large quantities.

Penrose (21) states that manganese replaces calcium in calcium carbonate and Murray and Irvine (20) advance this hypothesis to account for the formation of manganese nodules around calciferous organic remains.

Weidman (32) reports a pseudomorph of pyrolusite after calcite from northern Wisconsin. Here is a clear case of calcium carbonate precipitating manganese dioxide.

If solutions of manganous bicarbonate are percolated through columns of

calcium carbonate and the percolation is not continued too long, the manganese is almost quantitatively precipitated. After drying the calcium carbonate, dark particles, presumably manganese dioxide, can be detected in the mass. These dark particles decomposed hydrogen peroxide energetically.

Geloso (8) has found that colloidal manganese dioxide has the power to adsorb manganous salts. Tillmans, Hirsh, and Haffner (29) describe a process for the removal of manganese salts from water supplies. The water is filtered through manganese dioxide, which adsorbs the manganous salts, changing them into the insoluble dioxide. The formation of manganese concretions may be explained in this way. Once a particle of manganese dioxide is formed through the replacing of calcium, precipitation by bacteria (7), or any other cause, such a particle would continue to grow by adsorbing and fixing the manganous salts within its sphere of action.

In the soil, there are instances where the artificial addition of calcium carbonate has precipitated manganese. Schollenberger (24) found a black precipitate containing manganese on the surface of calcium carbonate particles picked out from pots of soil that had recently been limed. On some of the limed plots at the Rhode Island station plants suffer chlorosis which is supposed to be due in this case to lack of manganese (9). The precipitation and change of the exchangeable manganese to the insoluble dioxide would seem to be the explanation of this phenomenon. According to Truog (30) Masoni (16), Ruprecht and Morse (23), and Funchess (7), liming the soil makes the manganese insoluble or nearly so.

In the development of some soil profiles there has been a concentration of manganese in certain horizons. As a general rule, manganese is highest in the surface soil. It reaches a minimum in the B horizon and generally increases in the C horizon. Oxidation would favor the precipitation of manganese but in most soils manganese dioxide is not present. The manganese is possibly concentrated in the surface soil through the agency of plants. In many soils containing a layer of calcium carbonate below the surface, deposits of manganese dioxide are found just above the layer containing calcium carbonate. These deposits occur as well-defined concretions, as leaf-like deposits in soil cracks, and as a loose black powder in cavities formerly filled by calcium carbonate.

There is an interesting relation between the total manganese and the reaction of the horizons in soil profiles containing manganese dioxide. These relations are given for a few profiles in table 6.

The Blakely series in which manganese dioxide is present in the surface soil shows a correlation between the total manganese and the pH. In those soils the pH and percentage of manganese pass through a minimum in the B horizon and no manganese dioxide is found in the B horizon. A number of profile samples show the lack of manganese dioxide in the B horizon, but only two profiles, those shown in the table, extend into the C horizon. The Blakely series was originally formed from limestone but is deeply weathered and there

is no calcium carbonate in the soil profile. The results with the Blakely indicate that manganese dioxide is dissolved in the soil when the pH is below 5.0 or 5.2.

TABLE 6

Showing the relation between total manganese and pH in the soil material of certain profiles

SOIL SERIES	SOIL NUMBER	DEPTH	MnO	pH*
		inches	*per cent*	
Blakely clay loam, Daugherty Co., Ga.	30213	0–15	0.501	6.07
	30214	15–24	0.224	6.45
	30215	24–48	0.103	5.13
	30216	48–60	0.026	5.50
	30217	60–96	0.367	7.35
	30218	96–116	0.383	7.55
Blakely clay loam, Early Co., Ga.	32457	0–1½	0.53	6.87
	32458	1½–7	0.49	5.37
	32459	7–20	0.33	5.33
	32460	20–45	0.15	4.94
	32461	45–60	0.53	5.06
Blakely clay loam, Randolph Co., Ga.	258214	0–½	1.20	7.00
	258215	½–3	1.27	6.50
	258216	3–15	0.89	5.88
	258217	15–36	0.50	5.34
Labette silty clay loam, Crawford Co., Kan.	3821A	0–9	0.03	5.15
	B	9–14	0.05	5.13
	C	14–25	0.35	5.03
	D	25–35	0.30	5.19
	E	35–44	0.40	6.45
	F	44–60	0.09	7.75
Marshall silt loam, Doniphan Co., Kan.	3819A	0–13	0.12	5.88
	B	13–25	0.13	6.00
	C	25–32	0.16	6.09
	D	32–54	0.19	6.36
	E	54–62	0.11	7.80
	F	62+	1.03	7.79

* The pH determinations were made by E. H. Bailey of this Bureau.

The results on the Labette sample show that manganese has been carried down from the acid surface, but the precipitation apparently takes place in an acid horizon.

Well-defined manganese concretions occur in the Hockley and Edna silt loams in Victoria County, Texas. The concretions are found just above the carbonate layer, which is about 4 feet deep. The pH of the surface layers is about 6, rising to 7 and 8 where the concretions occur.

The Miami series, Miami County, Indiana, occasionally but not always show a few scale-like precipitates or soft concretions of manganese dioxide 3 or 4 feet below the surface. In this series the pH varies in the surface layers from 4 to 6 and reaches about 8 in the layer where the manganese occurs. Conrey and Schollenberger's (5) data show a similar correlation between manganese content and pH in the Clermont silt loam in Clermont County, Ohio.

SIGNIFICANCE OF MANGANESE DIOXIDE IN THE SOIL

Soils which have manganese dioxide in the A horizon have distinctive properties. They are rather sharply defined from contiguous soils containing no manganese dioxide. The color, a peculiar chocolate brown, is distinctive and unmistakable. The soil is loose, friable, well-drained, and in Hawaii generally contains less clay than adjoining soils (13). Pineapple plants growing on soils high in manganese dioxide frequently show chlorosis and many other plants do not develop normally on these soils. In our southern states, however, the Blakely soils, which are characterized by the manganese dioxide they contain, are markedly fertile.

Worsham et al. (31) have reported the presence of manganese dioxide in the "push land," a phase of the Davidson clay in Georgia. A great number of soils were tested for the presence of manganese dioxide with hydrogen peroxide.[2] All surface soils showing the presence of manganese dioxide had a peculiar chocolate brown color. These surface soils were confined to a very few series. Of these the Blakely series in Georgia and Alabama stood out prominently. It was found that this laboratory test would distinguish the Blakely from the contiguous Greenville and Orangeburg series. Some samples of the Davidson and Decatur surface soils gave the test. Lateritic soils from the Philippines, East Indies, and Cuba showed the presence of manganese dioxide in the surface soil.

Scale-like deposits and occasional concretions of manganese dioxide are occasionally found just above the horizon which contains limestone, as in the Fox, Miami, Bellefontaine, Clermont, Fillmore, and Vigo series. In general, however, the soil material in this particular horizon of the series as noted above does not give a positive test for manganese dioxide.

Well-defined concretions containing manganese dioxide occur in the lower horizons of the Hockley and Edna series in Texas and in the Scott series in Nebraska. These concretions occur just above the profile containing calcium carbonate.

In a phase of the Marshall silt loam in Troy, Kansas, 5 feet below the surface, manganese dioxide was present as a black, loose powder in cavities where limestone had been leached out.

Two profiles of the Greenville series showed relatively rich concentrations of manganese dioxide from 4 to 6 feet below the surface. The dioxide was

[2] Through the coöperation of Mark Baldwin, H. H. Bennett, W. T. Carter, W. E. Hearn, T. D. Rice, R. S. Roberts, and W. E. Tharp of the Soil Survey.

not present in well-defined concretions. One horizon contained 2.85 per cent MnO, whereas horizons just above and below contained only 0.03 per cent MnO. No calcium carbonate appears in the lower horizons of this series, but the series is considered as being derived from limestone and the precipitation of the manganese dioxide was probably caused by calcium carbonate.

Manganese concretions are found in the upper part of the Decatur profiles in northern Alabama. In the Shelbyville series in Kentucky, soft manganese concretions in the upper part of the C horizon are a characteristic feature of the series.

Two rough analyses of the colloidal matter from the Blakely series show a $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio of slightly less than 0.8. The analyses of lateritic soils containing manganese dioxide indicate a low $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio in the colloidal matter. A low silica-alumina-iron ratio is undoubtedly a characteristic of the colloidal matter in surface soils containing manganese dioxide.

SUMMARY

The presence of a small quantity of manganese dioxide in the soil causes the soil to decompose hydrogen peroxide so vigorously that the presence of manganese dioxide in the soil can be established by the hydrogen peroxide test. The test is not recommended for a quantitative determination of manganese dioxide, on account of probable differences to be found in the sizes of the manganese dioxide particles in different soils.

Manganese dioxide does not occur in the clay or colloidal fractions of soil. It occurs in the sands, but to a greater extent in the silt fraction. The determination of the total manganese in the silts and sands is an approximate determination of the manganese dioxide in the whole soil.

Concretionary and other deposits of manganese dioxide in the soil are apparently formed through the agency of calcium carbonate.

The presence of manganese dioxide in the surface layers of soil is characteristic of certain soil series. These soils are characterized by a peculiar and unmistakable chocolate brown color.

REFERENCES

(1) Ageno, F., and Valla, E. 1911 Hydrolysis. I. Hydrolysis of Carbonates. *Atti. R. Accad. Naz. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat.*, 20: 706–12.

(2) Bertrand, G. 1924 Sur le dosage des différentes formes du manganése contenues dans la terre arable. *Bul. Soc. Chim. Biol.* 35 (ser. 4): 1522–1527.

(3) Brewer, P. H., and Carr, R. H. 1927 Fertility of the soil as related to the forms of its iron and manganese. *Soil Sci.* 23: 165–173.

(4) Clarke, F. W. 1920 Data of Geochemistry. U. S. Geol. Survey Bul. 695.

(5) Conrey, G. W., and Shollenberger, C. J. 1927 The effect of weathering on exchangeable bases as shown in the Clermont silt loam profile. *First Internatl. Congr. Soil Sci.* 2 (Commission 2): 212–229.

(6) Doherty, M. W. 1898 Manganese nodules found at Onybygambali. *Australasian Assoc. Ad. Sci. Rpt.* 1898: 339.

(7) FUNCHESS, M. J. 1918 The development of soluble manganese in acid soils as influenced by certain nitrogenous substances. Ala. Agr. Exp. Sta. Bul. 201 (Tech. Bul. 4).

(8) GELOSO, M. 1927 Adsorption an sein des solutions salines par le bioxide de manganèse et généralités sur ce phénomène. III. Adsorption par le bioxide de manganèse colloidal. *Ann. Chim.* 7: 113–150.

(9) GILBERT, B. E., MCLEAN, F. T., AND HARDIN, L. J. 1926 The relation of manganèse and iron to lime induced chlorosis. *Soil. Sci.* 22: 437–446.

(10) GMELIN-KRAUT 1908 Handbuch der anorganischen Chemie, v. 3, part 2. Heidelberg.

(11) HELBIG, M. 1914 Neuer Untersuchungen Über Boden verkittung durch Mangan bezw. Kalk. *Naturw. Ztschr. Forst. u. Landw.* 12: 385–393.

(12) JOHNSON, M. O. 1924 Manganese chlorosis in pineapples; its cause and control. Hawaii Agr. Exp. Sta. Bul. 52.

(13) KELLEY, W. P. 1912 The function and distribution of manganese in plants. Hawaii Agr. Exp. Sta. Bul. 26.

(14) LOEW, O. 1901 Catalyse, a new enzyme of general occurrence. U. S. Dept. Agr. Rpt. 68.

(15) MCGEORGE, W. T. 1917 Composition of Hawaiian soil particles. Hawaii Agr. Exp. Sta. Bul. 42.

(16) MASONI, G. 1916 La reazione alcalina provocata dagli acidi Nelle terre. II. Solubilizyazione dei composti di manganese nel terreno. *Staz. Sper. Ag. Ital.* 49 (2): 132–149.

(17) MATTSON, S. E. 1926 Electrodialysis of the colloidal soil material and the exchangeable bases. *Jour. Agr. Res.* 33: 553–567.

(18) MAY, D. W., AND GILE, P. L. 1909 The catalyse of soils. P. R. Agr. Exp. Sta. Cir. 9.

(19) MERRILL, G. P. 1906 Rocks, Rock Weathering and Soils. New York.

(20) MURRAY, J., AND IRVINE, R. 1894 On manganese oxides and manganese nodules in marine deposits. Edinb. Roy. Soc. Trans. 37: 721–742.

(21) PENROSE, R. A. F. 1891 Manganese, its uses, ores, and deposits. *Geol. Survey of Ark. Ann. Rept.* 1890; (v. 1) pl. 642.

(22) ROSCOE, H. E., AND SHORLEMMER, C. 1923 Treatise on Chemistry, ed. 6, v. 2, New York.

(23) RUPRECHT, R. W., AND MORSE, F. W. 1917 The cause of the injurious effect of sulphate of ammonia when used as a fertilizer. Mass. Agr. Exp. Sta. Bul. 176.

(24) SCHOLLENBERGER, C. J. 1928 Manganese as an active base in the soil. *Soil Sci.* 25: 357–358.

(25) SHARRER, K. 1927 Zur Kenntnis der Hydroperoxid spaltenden Eigenshaft der Böden. *Biochem. Ztschr.* 189: 125–149.

(26) SUTTON, F. 1911 A Systematic Handbook of Volumetric Analysis. Philadelphia.

(27) THIEL, G. A. 1925 Manganese precipitated by micro-organisms. *Econ. Geol.* 20: 301–309.

(28) THRESH, MAY 1902 Manganiferous nodules in the boulder clay of Essex. *Essex Naturalist* 12: 137–139. *Jour. Chem. Soc.* 82: 567.

(29) TILLMANS, J., HIRSH, P., AND HÄFFNER, F. 1926 Removal of manganese from water supplies: 1. Absorption by manganese dioxide. *Gas. und Wasserfach.* 70: 26–30.

(30) TRUOG, E. 1916 The utilization of phosphate by agricultural crops, including a new theory regarding the feeding power of plants. Wis. Agr. Exp. Sta. Bul. 41.

(31) WORSHAM, W. A., JR., ET AL. 1918 Analyses of soils of Jasper County, Ga Agr. Exp. Sta. Bul. 160.

(32) WEIDMAN, S. 1907 Minerals from Northern Wisconsin. Wis. Geol. Nat. Hist. Survey Bul. 16.

AN EFFICIENT SOIL TUBE JACK

C. A. TAYLOR AND HARRY F. BLANEY

Bureau of Public Roads, United States Department of Agriculture

Received for publication January 16, 1929

During the past year the authors[1] have been conducting soil moisture studies is southern California, which require soil samples taken at 1-foot intervals to a depth of 18 feet. The success of the investigation depends upon the speed and economy with which a large number of samples can be obtained.

For the purpose of pulling the soil tubes, which are driven into the ground with a 30-pound hammer, a new type of jack was designed and perfected.[2] This jack is light and simply made, and is extremely effective in its operation. The manner of its operation is shown in plate 1. Figure 1 shows the soil tube, the jack when first placed in position, the hammer used in driving the tube, and the grip. Figure 2 shows the grip as first placed around the tube. Figure 3 shows the grip as it has been tightened around the tube and the jack in position for raising the tube.

The hinged grip which fits around the soil tube has a conical outer face. With this, two rollers fitted on prongs of the lever arm are in contact when the jack is operated. Force applied downward on the handle moves the two rollers upward along the face of the cone, causing the grip to take hold and draw the soil tube out of the ground. When pressure on the handle is released, the springs at the end of the forks return the lever arm to its original position. At the same time, the grip slips down the tube of its own weight since the pressure on its conical face is released. The base of the fulcrum has an area of 120 square inches, giving ample bearing surface on the ground. For the sake of lightness the base is made of cast aluminum and the complete jack with handle and grip weighs less than 23 pounds.

When the apparatus is in its ordinary position, a force of 150 pounds applied 30 inches out on the handle gives a pulling effort of 1,800 pounds on the soil tube. This effort may be changed at will by applying the force on the handle at different positions or by moving the base so that the distance from the fulcrum of the point of contact of rollers and cone is varied.

Two of these jacks have been in operation for the past six months and have been put to severe tests, pulling tubes out of loose gravelly sand and also from

[1] Under the supervision of W. W. McLaughlin, Associate Chief of the Division of Agricultural Engineering, Bureau of Public Roads, United States Department of Agriculture,

[2] In coöperation with H. E. Twomley, designing draftsman of the Parker Machine Works, Riverside, California.

tight dry clay. In several cases, the pulling effort on the soil tube was estimated to be over 4,000 pounds. The jacks have pulled tubes wherever it was possible to drive them with 30-pound hammers. Jacks of this type cost $30 each.

PLATE 1

FIG. 1. FIRST POSITION OF JACK, HAMMER, AND GRIP
FIG. 2. GRIP AS FIRST PLACED AROUND THE TUBE
FIG. 3. JACK IN POSITION FOR RAISING TUBE

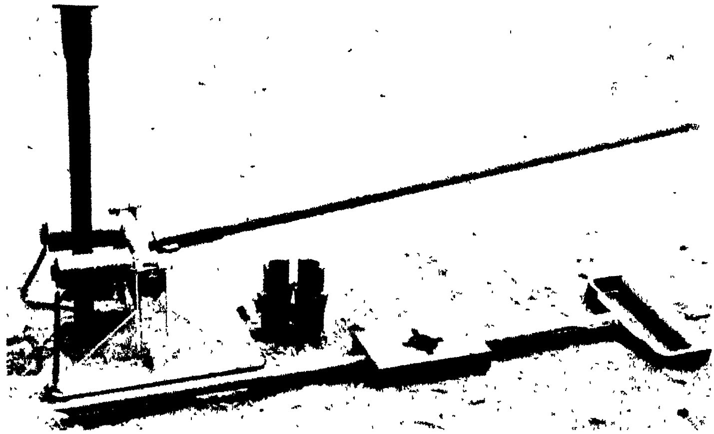


FIG. 1

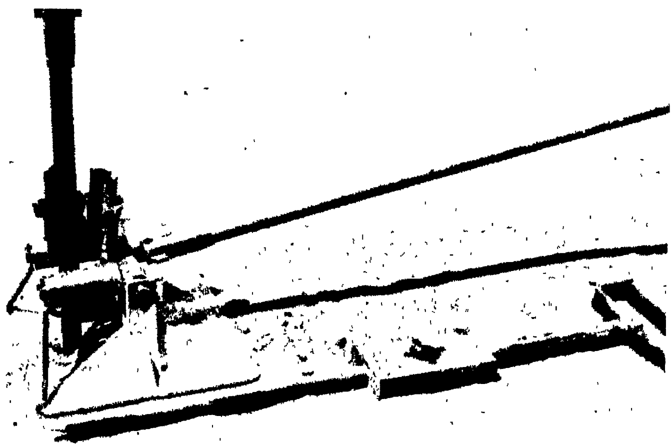
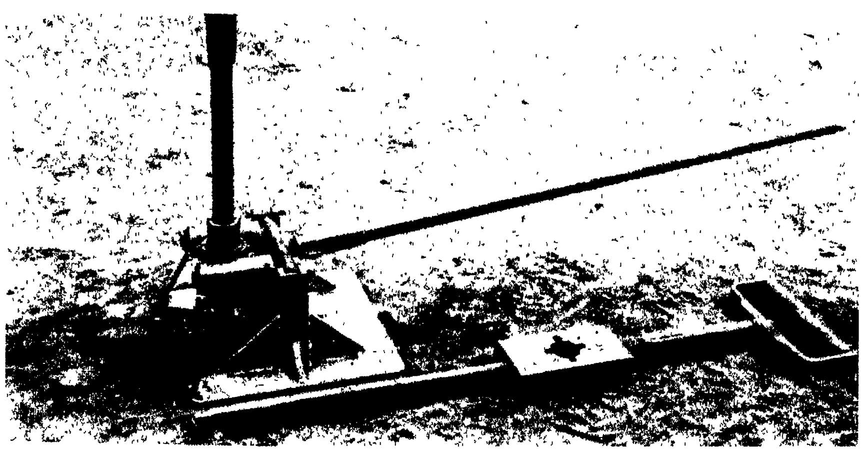


FIG. 2



SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: II. INFLUENCE OF THE STAGE OF PLANT GROWTH UPON ABUNDANCE OF ORGANISMS

ROBERT L. STARKEY

New Jersey Agricultural Experiment Station

Received for publication January 22, 1929

The introductory material presented in the preceding report (2) demonstrates that development of plant roots leads to marked modification of the biological condition of the soil near the developing roots. This modification appears in the greater abundance of all groups of the microörganisms studied and also in the evolution of carbon dioxide from the soil. These results indicate merely that the modification of the soil population was apparent at the particular period of plant growth at which the studies were made. They give no idea as to whether the effects are the same throughout the growth of the plants or at what periods of plant growth the most marked effects may appear. Smith (1) observed that the growth of legumes increases the numbers of *B. radiobacter* beginning the third week and extending some weeks after harvesting the crop. Wilson and Lyon (3) grew pure cultures of organisms in soils in the presence and absence of plant growth. Although the results indicated that the numbers of bacteria are greater under plant growth, there was little to indicate that there is any consistent difference in the extent of the influence of the plants upon the organisms at different stages of plant development.[1]

The following studies were planned with the object of determining more conclusively the extent of the influences of different plants upon the soil population and particularly what differences might be exerted by the plants at different stages of growth.

EXPERIMENTAL PROCEDURE

The experimental studies were conducted upon soils in the greenhouse and in the field. The soil used for the greenhouse work was a fairly heavy acid soil (pH 5.5) being a natural mixture of Penn loam and Sassafras loam. This proved to be too heavy for best results. Although there was fairly good growth of most of the experimental plants, corn failed to make normal development. The plants used in the greenhouse studies were: oats, beans, rape, table beets, and sweet clover. Such plants were selected as would show a

[1] An extensive historical introduction to this subject may be found in part I of this series (2).

variety of characteristics of root development: fibrous roots, fleshy roots, tap roots, spreading roots, annuals, and biennials. There were 36 pots of soil involved in these studies, 6 planted to each of the five plants used and 6 left unplanted. Each pot contained 20 pounds of soil.

At each period of sampling, one pot of soil growing each of the plants and one unplanted pot of soil were used. The tops of the plants were harvested, the soil was then removed from the pots, the roots were separated as completely as possible from the soil, and then samples of the soil were passed through a 3 mm. sieve and brought to the laboratory for examination.

The legumes were inoculated at the time of planting. After all of the plants were well started they were thinned out to leave ten plants in each pot of oats, rape, and sweet clover; nine of beets; and six of beans.

The soils were kept close to the optimum moisture content throughout the experiment. Weeds were removed from the soil as they made their appearance. Since the soils were deficient in available nitrogen at the first period of sampling (36 days after planting) 2 gm. of sodium nitrate was added to each of the pots of soil except those supporting legumes.

The field studies were conducted upon a Sassafras loam soil of neutral reaction. The physical condition of this soil was good and brought about excellent development of all the plants which were used. The plants used in the greenhouse studies (oats, beans, rape, table beets, and sweet clover) were also used in the field and, in addition, potatoes, field corn, and mangel beets. Because the sweet clover was planted somewhat later than the other plants, differences will appear in the results in the recorded age of the sweet clover at the periods of sampling as compared with the other plants. The legumes were inoculated at the time of planting.

After the plants showed fair development they were thinned out sufficiently to prevent crowding. Throughout the season, weeds were eliminated by pulling by hand. One portion of the plot was left unplanted but otherwise treated identically as the planted soils. This unplanted section was used as a source of samples of soil supporting no plant development for comparison with samples of soil collected from about the roots of the various plants. In sampling the soils, the plants were carefully removed with some soil clinging to the roots. The soil was removed from the roots and samples were passed through a 3-mm. sieve and brought to the laboratory for study. Records were kept of the vigor and stage of growth of the plants as well as of the weights of the tops and root systems at each period of sampling.

The soils collected from the unplanted section and from the roots of the plants were examined for the abundance of filamentous fungi, actinomyces, bacteria developing upon albumin agar, organisms developing upon nitrogen-free mannite agar, and mucoid colonies (*B. radiobacter*) developing upon nitrogen-free mannite agar as outlined in the preceding report (2). The abundance of *B. radiobacter* was also determined by plating the soils upon glycerol-nitrate-soil extract agar as suggested by Smith (1). This method was slightly modified

in that just before pouring the plates, the crystal violet was added to the melted agar (in 300 cc. portions in 500 cc. Erlenmeyer flasks) in such amounts as to create a concentration of 1 part of dye to 100,000 parts of medium. Smith added the dye to the soil suspensions before adding them to the plates. An attempt was also made to determine the abundance of nitrogen fixing organisms. This involved the introduction of 1-cc. portions of dilutions of the soils

TABLE 1

Influence of development of higher plants upon abundance of bacteria (1/1,000,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	34.2	14.6	30.4	19.8	17.0	23.2
Oats	33.6	42.0	88.0	34.6	29.2	45.5
Corn	40.4	29.8	42.6	48.0	37.0	39.6
Beans	26.7	26.0	47.8	51.8	42.8	39.0
Potatoes	36.0	23.4	38.0	21.3	14.8	26.7
Table beets	37.2	22.6	60.4	41.4	47.8	41.9
Mangel beets	44.7	38.4	48.0	48.6	51.8	46.3
Rape	71.2	46.8	43.6	69.4	88.4	63.9
Sweet clover*	34.8	27.2	29.2	33.0	50.2	34.9

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	28.3	34.6	19.3	21.4	17.4	9.0	21.7
Oats	30.8	20.5	31.2	50.0	36.2	31.4	33.4
Beans	36.6	45.3	22.0	28.2	15.8	12.4	26.7
Beets	26.5	57.5	19.8	31.7	42.0	13.6	31.9
Rape	21.8	93.7	46.5	157.6	24.6	19.6	60.6
Sweet clover	35.4	36.8	36.0	21.8	29.0	9.8	28.1

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

in duplicate into flasks containing 100-cc. portions of nitrogen-free dextrose solutions. Similar determinations conducted in mannite solutions indicated the presence of much smaller numbers than did the dextrose medium. Records were kept of the dilutions which resulted in fixation of nitrogen and those from which no fixation occurred after 30 days.

EXPERIMENTAL RESULTS

Numbers of bacteria

Table 1 and figure 1 indicate the results of the enumeration of bacteria. In the table the results of both the field and greenhouse study are included;

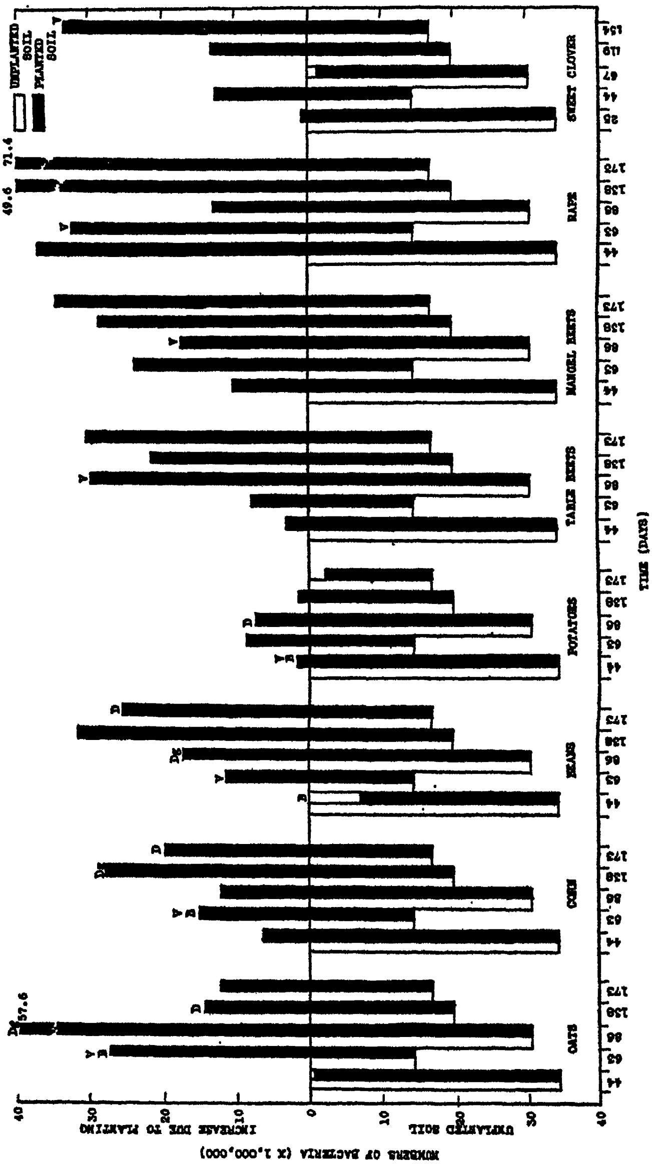


FIG. 1. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF BACTERIA IN THE SOIL

only the field results are presented graphically since the two sets of data are much the same. The plant growth was so much more vigorous and normal in the field that the results from studies of these plants should be much more characteristic than those of the greenhouse plants. In the greenhouse all of the plants except the oats became quite stunted during the last periods of the extended study. It is also apparent that, in soils kept for long intervals, soluble salts may accumulate to concentrations far greater than would occur in the field in humid regions.

In the figure, the unshaded columns extending below the zero line represent the abundance of bacteria in the fallow soil. The black columns represent the abundance of bacteria in the soil obtained from the rhizosphere of the various plants. Since the black columns extend below the zero line to the point of the unshaded columns any extension of the black columns above the zero line represents the detection of greater numbers of bacteria in the planted soil. Likewise, if the black columns do not extent to the zero line it indicates that the numbers found in the planted soil were smaller than those found in the unplanted soil.

Since most of the black columns extend above the zero line it is apparent that the development of higher plants has in practically all cases increased the abundance of bacteria which are detected by this plate method. In some cases the increases are much more pronounced than in other instances. More than this, there are certain differences which may be ascribed to the difference in type of development of the various plants and, further, some of these variations may be correlated with the stages of growth of any one plant. It is to be expected that plants showing such a variety of growth characteristics (fibrous and fleshy roots, tubers, annuals, biennials, legumes, and non-legumes) would affect the soil population differently. In general, these results show that the influences of most of the plants are not great in the early stages of plant growth and that the maximum effects generally appear only after the plants have attained considerable size, have reached the height of vegetative growth, bloomed, or started vegetative degeneration. Subsequent to the death of the plants there is generally a pronounced decline in the abundance of bacteria.

The letters written above the columns on the figure indicate the stages of plant development at the different periods of sampling the soils (*B*—blooming, *V*—height of vegetative development, *Dg*—initial degeneration, *D*—death).

Under the potatoes which mature and die within a short period of time there is a marked increase in numbers after the early stage of growth and pronounced decline subsequent to maturity and death of the plants. A similar striking change is observed under the oats. With the corn and beans the decline after death is not as apparent since the plants were killed by frost just before the last sampling and the interval between death and sampling was too short for the biological activities to drop to a low level. The biennials show a very marked effect upon the organisms even at the last periods of sampling. At this time these plants were in active development while the annuals were all

dead. This difference between the annuals and biennials does not appear to be as striking with the greenhouse soils as with the field soils, presumably because of the degenerated condition of all the plants in the greenhouse at the late periods.

This periodic influence of the plants may logically be considered as being correlated with the periods of youthful root development accompanied by little

TABLE 2

Influence of development of higher plants upon abundance of organisms developing upon nitrogen-free mannite agar (1/1,000,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	33.3	26.2	17.8	8.0	12.2	19.5
Oats	28.6	44.2	52.6	13.6	15.0	30.8
Corn	39.0	34.8	34.6	23.2	21.0	30.5
Beans	39.8	31.2	33.6	25.4	30.4	32.1
Potatoes	31.0	42.8	29.8	13.0	15.8	26.5
Table beets	45.4	31.5	49.0	19.8	32.2	35.6
Mangel beets	65.8	38.8	36.6	22.8	34.4	39.7
Rape	81.2	70.8	46.8	18.6	45.4	52.6
Sweet clover*	35.7	33.4	26.0	16.4	23.2	26.9

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	13.8	14.0	6.4	5.0	9.4	11.0	9.9
Oats	21.0	14.0	19.4	28.4	23.6	30.2	22.8
Beans	29.8	30.0	12.8	19.4	12.4	14.3	19.8
Beets	16.3	21.2	9.2	27.4	21.4	23.3	19.8
Rape	20.0	49.8	19.0	105.4	15.6	23.2	72.2
Sweet clover	26.6	32.2	17.6	21.0	17.4	16.6	21.6

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

root excretion of microbial foods, of periods of active root excretion of considerable amounts of material available to the microörganisms as food, and finally of root degeneration and death with only the more resistant materials becoming available to the microörganisms with no further replacement of these substances by the plant.

It may be noted that there appears to be a general tendency for the bacteria in the unplanted soils to decrease throughout the period of study. This might be expected since, in a fallow soil, the readily decomposable organic

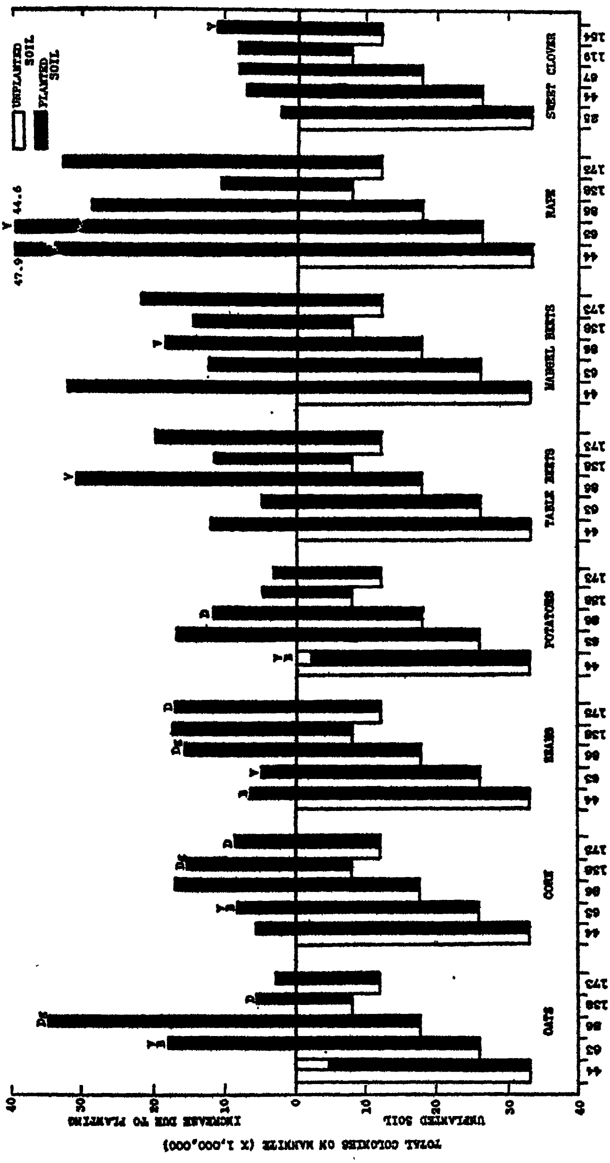


FIG. 2. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL WHICH GROW UPON NITROGEN-FREE MANNITE AGAR

matter is becoming continuously depleted and microbial foods are consequently less abundant. The marked effect of rape upon the bacteria is particularly striking and appears also in the studies of the other groups of organisms, as will be seen in the following pages. It is equally apparent that there is little correlation between the sizes of the plants and their effects upon the bacteria since the oats exerted greater effects than the field corn, and the beans produced

TABLE 3

Influence of development of higher plants upon abundance of B. radiobacter *group developing upon nitrogen-free mannite agar (1/100,000)*

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	9.8	9.4	3.8	1.0	3.4	5.5
Oats	6.6	31.4	19.2	2.6	7.0	13.4
Corn	12.8	18.4	8.0	3.8	8.6	10.3
Beans	13.6	15.0	6.8	9.0	14.0	11.7
Potatoes	11.2	11.4	5.2	2.2	5.8	7.2
Table beets	10.2	10.4	12.0	3.2	10.6	9.3
Mangel beets	20.8	17.2	9.0	7.8	14.8	13.9
Rape	31.8	27.0	11.6	8.4	12.0	18.2
Sweet clover*	7.2	16.6	7.0	3.6	12.4	9.4

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	8.8	5.4	3.4	1.4	0.6	2.4	3.7
Oats	16.2	10.5	16.0	9.8	16.0	21.2	15.0
Beans	21.0	19.6	16.5	10.2	5.0	10.0	13.7
Beets	8.6	12.3	6.4	8.3	8.0	7.0	8.4
Rape	15.4	20.0	17.3	10.0	9.3	9.4	13.6
Sweet clover	17.4	16.5	12.0	10.8	10.2	5.5	12.1

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

effects comparable to the corn. These three plants differed very greatly in amounts of root systems, vegetative growth, and seed development.

Colonies on mannite

The influences of plant development upon the abundance of organisms which grow on nitrogen-free mannite agar are shown in table 2 and figure 2. The organisms developing upon this medium are much the same as those appearing upon the albumin agar and, as might be expected, they manifest much

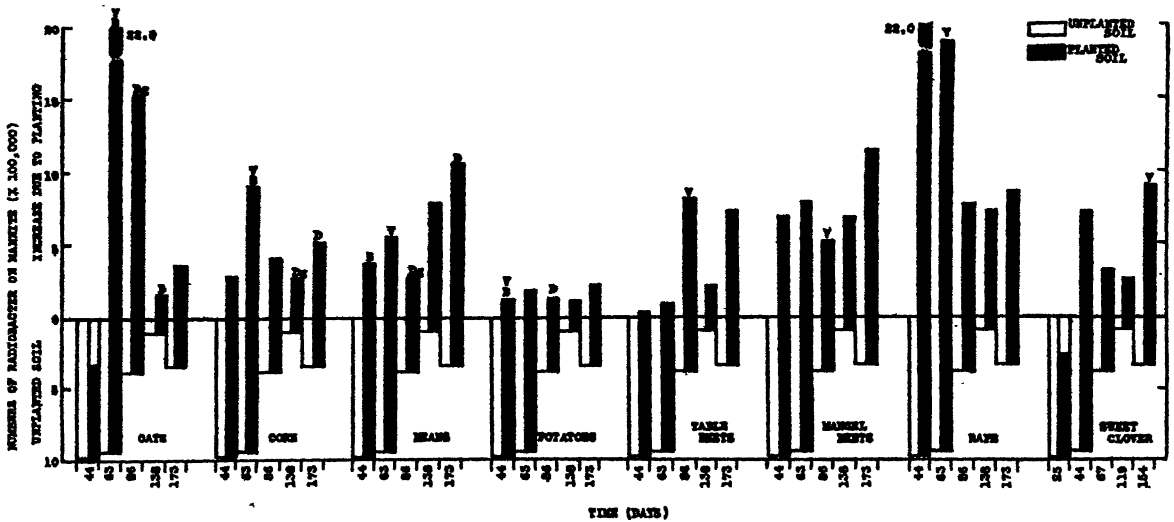


FIG. 3. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL OF THE *B. radiobacter* GROUP WHICH GROW UPON NITROGEN-FREE MANNITE AGAR

the same responses to plant development. These responses appear in the relatively low numbers of organisms at early stages of plant growth (except with the mangel beets and rape), maximum numbers in most cases at advanced periods of plant growth, depression in numbers subsequent to death of the plants, relatively high numbers at advanced periods in growth of biennials as compared with annuals and, in all observations, much greater numbers of or-

TABLE 4

Influence of development of higher plants upon abundance of B. radiobacter *group developing upon glycerol-nitrate agar (1/10,000)*

Field soils

PLANT	AGE OF DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	5.4	9.2	9.0	3.2	4.2	6.2
Oats	7.8	78.0	63.2	8.6	6.7	32.9
Corn	6.8	20.2	31.8	33.4	9.6	20.4
Beans	19.8	25.4	44.0	3.6	16.4	21.8
Potatoes	7.8	9.8	53.4	12.0	5.0	17.6
Table beets	8.4	15.4	35.6	21.8	13.8	19.0
Mangel beets	14.0	64.0	31.6	40.0	14.0	32.7
Rape	466.0	86.0	63.6	51.2	36.4	140.6
Sweet clover*	11.4	20.0	19.0	6.2	28.2	17.0

Greenhouse soils

PLANT	AGE OF DEVELOPMENT					AVERAGE OF ALL PERIODS
	59 days	93 days	128 days	169 days	202 days	
Fallow	15.0	9.0	1.2	1.0	0.4	5.3
Oats	6.0	13.3	6.2	48.2	18.8	18.5
Beans	36.6	20.6	51.2	9.2	8.4	25.2
Beets	13.8	8.6	10.8	2.8	8.4	8.9
Rape	31.0	22.4	27.4	2.6	3.2	17.3
Sweet clover	17.4	13.6	24.6	18.4	4.6	15.7

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

ganisms in soils near plant roots as compared with the abundance in unplanted soils. As with the bacterial counts, the numbers of the organisms show a progressive decrease in the unplanted soil as time elapses. There is a striking difference in the influences of the different plants upon the organisms; the most marked effect appears under the rape and the most transitory effect under potatoes. The explanation for these apparently characteristic effects of different plants still remains to be determined.

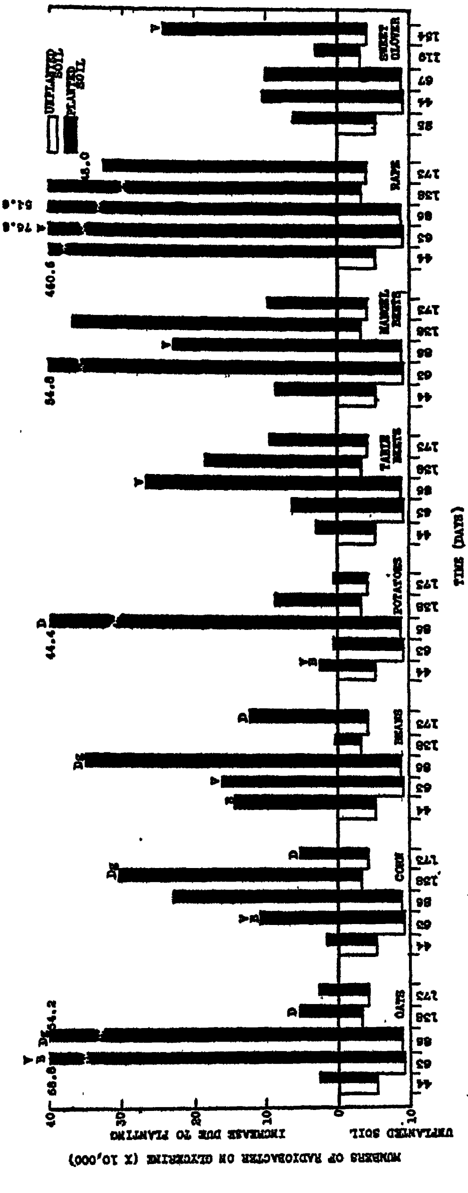


FIG. 4. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL OF THE *B. radiobacter* GROUP WHICH GROW UPON GLYCEROL AGAR

B. radiobacter group developing upon mannite

As seen by table 3 and figure 3, the organisms producing mucoid colonies upon nitrogen-free mannite agar (*B. radiobacter group*) also increased about the plant roots. In fact the proportional increases are somewhat greater than appeared in the organisms previously considered. The oats and rape showed the most pronounced increase. The potatoes showed comparatively slight influence upon this group of organisms at any stage of growth. The

TABLE 5

Influence of development of higher plants upon abundance of actinomyces (1/1,000,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	12.8	7.0	12.0	7.4	10.2	9.9
Oats	10.8	6.4	10.0	10.6	10.8	9.7
Corn	11.6	7.6	11.0	13.0	8.0	10.2
Beans	14.3	8.2	15.4	11.0	10.4	11.9
Potatoes	9.7	10.0	11.2	14.0	10.0	11.0
Table beets	10.3	9.0	17.0	12.3	14.2	12.6
Mangel beets	19.0	9.0	16.2	11.2	17.2	14.5
Rape	12.0	11.5	9.2	9.8	10.8	10.7
Sweet clover*	10.0	10.0	13.3	8.0	12.0	10.7

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	5.5	7.0	4.5	5.8	3.0	3.8	4.9
Oats	4.6	3.8	3.6	6.4	4.8	7.2	5.1
Beans	6.0	8.3	5.0	6.8	5.8	5.4	6.2
Beets	5.0	7.5	4.8	4.8	4.2	4.6	5.2
Rape	5.0	16.8	7.5	11.6	4.8	5.4	8.5
Sweet clover	7.4	7.0	8.0	7.6	3.8	4.0	6.3

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

beans showed an unusually pronounced effect upon these organisms at the final stages of growth. In general, the course of the changes in the numbers of these organisms in both the planted and fallow soils appear much the same as in the organisms previously considered.

B. radiobacter group developing upon glycerol

The results concerned with this group appear in table 4 and figure 4. Pronounced increases in the abundance of these bacteria appeared under the

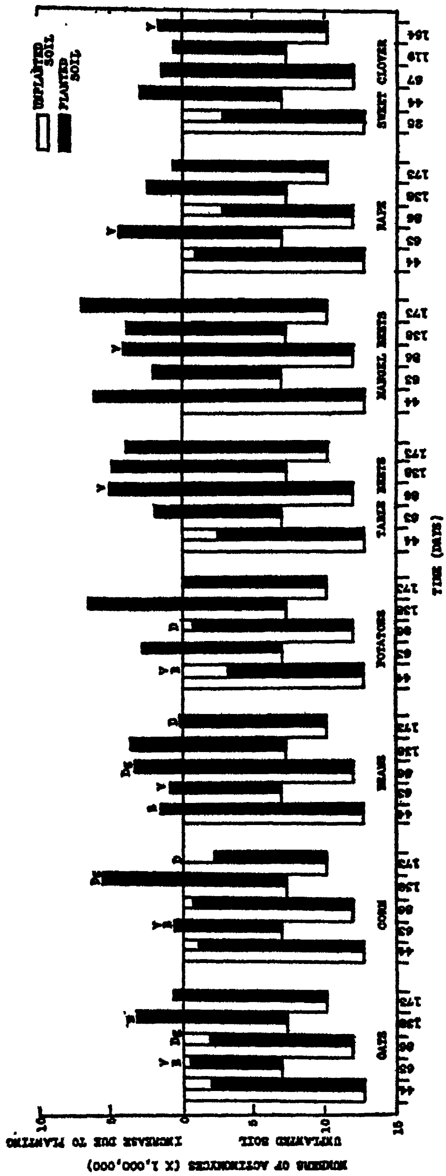


FIG. 5. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ACTINOMYCES IN THE SOIL

developing plants. The proportional increases are more pronounced than those observed with any of the other groups of organisms which were studied. Here the influence of rape is particularly great. The organisms from soil growing this plant reached the point of 4.6 millions, while the unplanted soil at the same period showed the presence of only 54 thousands. However, the absolute increases in most cases are much lower than with the other organisms previously considered since this group of bacteria makes up a much smaller portion of the

TABLE 6

Influence of the development of higher plants upon abundance of filamentous fungi (1/10,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	16.8	9.0	13.0	27.0	23.2	17.8
Oats	18.2	21.6	26.8	21.0	19.4	21.4
Corn	14.0	18.6	16.0	28.0	28.0	20.9
Beans	24.6	16.0	23.8	22.8	31.4	23.7
Potatoes	24.8	13.6	17.6	29.0	19.4	20.9
Table beets	11.8	15.3	22.6	18.8	25.4	18.8
Mangel beets	23.8	18.5	27.8	18.6	26.6	23.1
Rape	14.4	16.3	12.2	28.4	28.6	20.0
Sweet clover*	16.0	16.2	16.4	16.2	21.2	17.2

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	19.6	21.2	14.0	23.0	16.6	16.2	18.4
Oats	25.8	13.8	20.0	20.0	12.2	22.6	19.1
Beans	18.8	23.2	19.2	19.0	16.0	10.4	17.8
Beets	20.2	17.8	21.8	26.2	21.6	21.8	21.6
Rape	20.6	28.6	17.2	18.6	18.4	13.4	19.6
Sweet clover	21.0	19.0	20.6	14.0	22.8	14.2	18.6

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

total soil population than the other groups. In view of the pronounced response of these bacteria to plant growth it would be of considerable interest to ascertain what factors are responsible for this change.

The qualitative differences between the influences of the annuals and biennials are less pronounced upon these organisms than upon the general bacterial population of the soil. With the exception of the soil supporting sweet clover the numbers of the *B. radiobacter* group decreased at the late periods of plant development in all cases.

Numbers of actinomyces

The results of the observations of the actinomyces presented in table 5 and figure 5 show that this group of organisms is affected by plant development very differently than are the other groups of organisms previously studied. The increases are generally erratic and show little or no correlation with the stages of plant development. Likewise their abundance in the unplanted soil shows little definite change during the season. If they are influenced by the plant development, the method used for their study has not detected the effect. At the most, it might be conceded that the development of plants has increased the abundance of actinomyces about the root systems but that this increase is comparatively slight. Of 70 cases observed (40 from field soils and 30 from greenhouse soils), 50 showed greater numbers of actinomyces in the planted soils than occurred in unplanted soils, while 19 showed fewer actinomyces in the planted soils.

Numbers of filamentous fungi

The group of filamentous fungi show as little variation in numbers which may be interpreted as response to plant growth as do the actinomyces. These results are presented in table 6 and figure 6. The numbers generally appear to be somewhat greater at the stages of extensive growth and are quite low in soils supporting annuals subsequent to degeneration of the plants. However, it is difficult to explain the low level of fungus numbers at the late stages of development of the biennials in case one accepts as a fact that such a method of studying the fungi reflects the abundance of their occurrence in the soil.

In the light of our present understanding of the activity of filamentous fungi in soils it seems logical to conclude that these results do not properly indicate the extent of influence of plant development upon this group of organisms. Although it appears that the fungi were more abundant near the plant roots, the method of determination is so unsatisfactory that, unless pronounced differences are obtained, it seems unwarranted to interpret the differences as being more than suggestive. In the 70 cases studied, 45 showed greater numbers of fungi about plant roots and 25 showed greater numbers away from the roots. The unplanted soils showed no consistent change in numbers of fungi during the period of study.

Numbers of organisms fixing nitrogen

As outlined with the experimental methods these organisms were estimated by inoculating diluted suspensions of soil into flasks of nitrogen-free nutrient solutions. As each period of sampling, the suspensions were inoculated into flasks in duplicate at each of five dilutions including the limits of growth of the organisms. It frequently occurred that organisms would develop at certain high dilutions of the series with no growth at some of the lower dilutions, causing "skips" in the series. With only two cultures at each dilution and so many "skips" in the series it is not possible to determine the number of nitrogen-

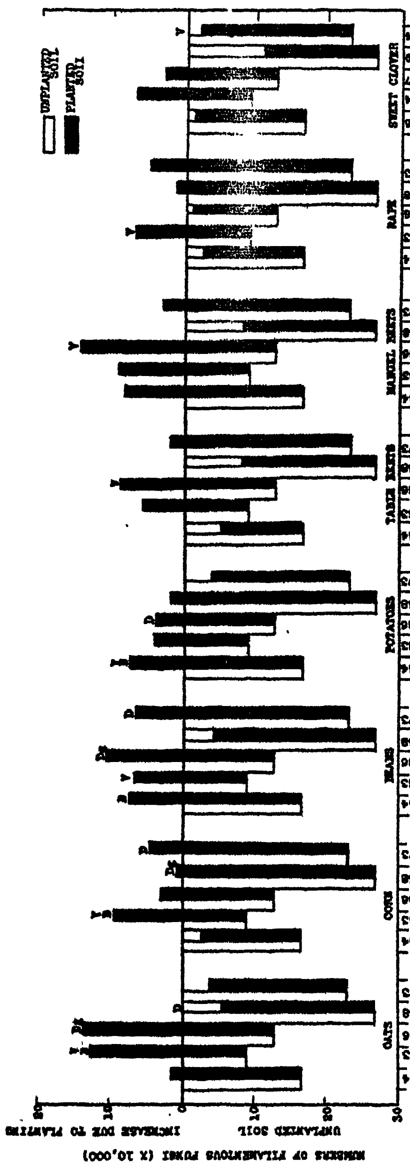


FIG. 6. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF FILAMENTOUS FUNGI IN THE SOIL

fixing organisms with any degree of accuracy. In order to suggest the general trend of the results, however, a summary is presented in table 7. These data present merely a list of the number of cultures in each series at each dilution which showed fixation of nitrogen. In the series of studies of the greenhouse soils a mannite medium was used but this failed to support development of nitrogen-fixing organisms at dilutions of the soil greater than 1,000. With the

TABLE 7

Numbers of cultures showing nitrogen fixation from planted and unplanted soils

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Cultures used	10	10	10	10	10	10
Fallow	6	6	7	3	5	5.4
Oats	8	8	7	4	3	6.0
Corn	8	6	6	5	7	6.4
Beans	8	5	6	5	6	6.0
Potatoes	6	7	5	4	5	5.4
Table beets	8	8	4	7	5	6.4
Mangel beets	8	7	7	7	7	7.2
Rape	8	5	5	6	5	5.8
Sweet clover*	8	6	7	3	5	5.8

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Cultures used	10	10	10	10	10	10	10
Fallow	4	3	4	5	5	6	4.5
Oats	3	2	9	7	5	8	5.7
Beans	3	2	5	5	5	6	4.3
Beets	4	4	6	7	7	6	5.7
Rape	2	3	4	7	7	9	5.3
Sweet clover	3	2	7	9	3	7	5.2

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

field soils a dextrose medium was used and nitrogen fixation occurred quite regularly at dilutions of the soils of hundreds of thousands and frequently of millions. It is quite apparent that, irrespective of the medium used, the type of plant developing upon the soil, or the stage of development of the plants, there is nothing which appears sufficiently different from the results from unplanted soils to suggest that plant growth appreciably modified the abundance of nitrogen-fixing organisms in the soil. Although these organisms appeared

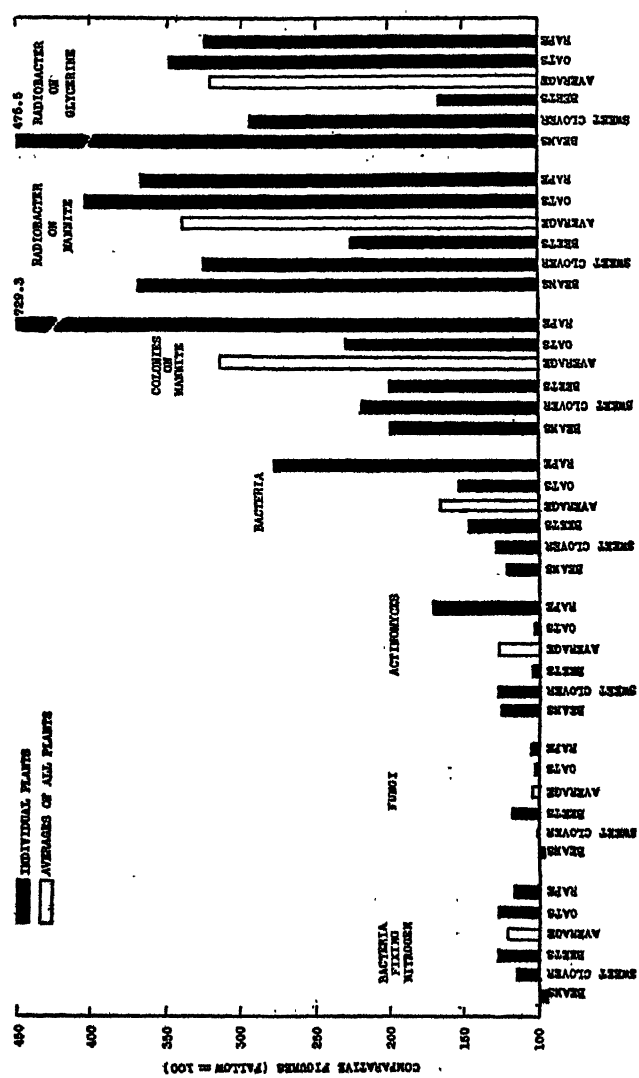


FIG. 7. AVERAGES OF THE GREENHOUSE STUDIES OF THE INFLUENCES OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL

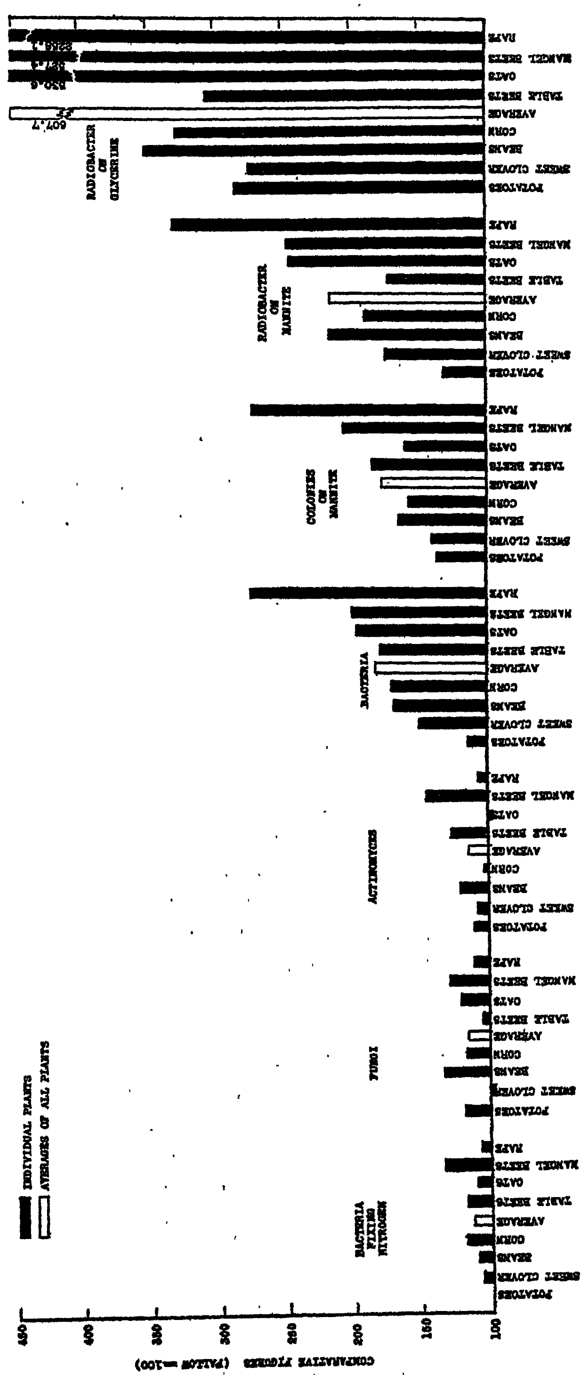


FIG. 8. AVERAGES OF THE FIELD STUDIES OF THE INFLUENCES OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL

to the extent of hundreds of thousands no consistently greater number of these bacteria developed from soils obtained from the rhizosphere than from soil obtained from a distance from the roots. Further, the legumes showed no more effect upon these organisms than did the non-legumes. In view of the plausable theoretical possibility that the rhizosphere may be a suitable habitat for development of nitrogen-fixing bacteria it appears strange that no results were obtained which would verify this supposition. The possibility still remains that the method which was used was unsuited to determine the abundance of this group of organisms.

Averages of influences of plants upon all the groups of organisms

In figures 7 and 8 the data are presented as averages of the determinations at all periods of sampling. Comparative figures are given, the results from the unplanted soil being considered as 100. The unshaded columns are averages of the average effects of all the plants upon each of the groups of organisms. In each group, the data are arranged from left to right in the order in which the different plants affect the abundance of bacteria in the soil.

It is evident that plant development exerts very different proportional effects upon different elements of the soil population. The apparent effects upon the nitrogen-fixing organisms and upon the fungi and actinomyces are very slight. The bacterial population as a whole and the organisms developing upon mannite agar are affected to a marked degree and in much the same order with certain few exceptions. The bacteria of the Radiobacter group increased to a much greater proportional degree than any of the other organisms. This is particularly striking with the results of their detection upon glycerol media from the field soils. Among all of the data, the striking influence of rape upon all the groups of soil organisms is clearly apparent. It was expected that the legumes would effect a somewhat more marked influence upon the soil population than is apparent from these results in view of the fact that it has been repeatedly claimed that such plants have the most pronounced influence upon soils. Since, at all the periods of study, the sweet clover plants were less mature than any of the others the comparison of their effects during early stages of growth does not accurately indicate their influence upon the soil organisms over extended periods of time.

If the order of the influence of the plants upon the numbers of bacteria in the soil during the growing season was the same for all of the biological groups, it might be assumed that this order of the plants represented the order of their influences upon the microbial population as a whole. In some cases this appears to be the case, but it appears more logical in many cases to assume that the order of influence of a group of plants upon one group of soil organisms may not be the same upon all soil organisms. These influences of different plants may be so markedly altered by cultural conditions which modify the extent of plant growth, the period of time before maturity, and other physiological characteristics of plants that any attempt to indicate without qualifi-

cation what plants exert the greatest or slightest effects upon the soil organisms would be replete with errors. It is more desirable to ascertain what factors associated with plant growth are responsible for this effect upon the soil organisms.

TABLE 8

Correlation between weights of roots of single plants at the periods of sampling the soils and the influences of the plants upon the abundance of bacteria in the soil

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE INCREASE IN NUMBERS OF BACTERIA DUE TO PLANT GROWTH
	44 days	63 days	86 days	138 days	173 days	*millions*
Potatoes	3.36	8.91	25.1*	28.3†	51.0†	3.5
Sweet clover‡	0.003	0.08	0.6	10.5	61.0	11.7
Beans	0.47	0.66	0.7	2.1	1.2	15.8
Corn	3.36	12.26	38.0	48.0	41.0	16.4
Table beets	0.35	5.8	21.8	28.5	22.5	18.7
Oats	0.25	0.31	0.3	0	0	22.3
Mangel beets	0.34	7.1	26.5	90.3	77.3	23.1
Rape	0.99	1.20	2.0	6.8	7.7	40.7

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE INCREASE IN NUMBERS OF BACTERIA DUE TO PLANT GROWTH
	59 days	93 days	128 days	169 days	202 days	*millions*
Beans	0.136	0.068	0.085	0.131	0.069	5.0
Sweet clover	0.002	0.006	0.247	0.252	0.314	6.4
Beets	0.008	0.071	0.298	0.511	0.315	10.2
Oats	0.024	0.080	0.304	0.341	0.124	11.7
Rape	0.015	0.073	0.414	0.541	0.420	38.9

* Mainly tubers only 0.56 gram fibrous roots.
† All tubers, no fibrous roots.
‡ For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

Influence of extent of root systems

It might be expected, since it is through the root systems of the higher plants that the influences become exerted upon the soil organisms, that there would be some correlation between the abundance of the roots and the extent of the changes in the organisms in the soil. An inspection of table 8 indicates quite definitely that there is little or no correlation between the weights of the plant roots and the average increases in bacteria in soils under the plants. There is no greater correlation between the weights of roots at any one period of

sampling and the abundance of bacteria under the plants at that period. Further, no apparent correlation exists between the root weight and the abundance of any of the other organisms which were studied.

It may not seem justified to compare the weights of roots of single plants since different numbers of plants covered equal areas of soil. It would seem unlikely, however, that any comparison of weights of roots occupying equal volumes of soil would show correlated relationships between the effects of these roots and the soil population. It might be logical to assume that correlations would exist between the extent of root surfaces and the influences of the plants in any unit volume of soil. It seems more likely that none of these factors would have more than a chance relationship with the observed effects. Other factors related to the physiological behavior of the plants undoubtedly control the reactions. It seems apparent that young roots exert comparatively slight effects and that, at the period close to fruiting or at advanced stages of growth, the plants exert the most marked influences. This would appear to suggest that factors associated with the quality and quantity of root excretions (particularly organic) might be factors of major importance in this connection. These are related to certain characteristics inherent in the plants themselves and would not justify an assumption that the abundance of root systems would be of most importance in determining the observed changes.

DISCUSSION

It is quite apparent that the plate method of determining the abundance of organisms in the soil is far from satisfactory. However, the results which have been obtained appear of such an order as to indicate that the apparent effects of the plants upon the soil organisms are significant effects in most cases. The fact that marked effects of plant growth upon the soil organisms were observed by the methods which were used may suggest that even more marked effects are actually brought about by the plants. This would seem likely since the large samples of soil which were taken for analysis necessitated the collection of considerable soil which was not in contact with the plant roots. If it is assumed that root excretions are of major importance in determining the results, the greatest effects should appear closest to the roots. Further studies of more detailed nature of conditions in the zone of the root surfaces should indicate that the microörganisms are even more profoundly modified by plant growth than the present results would suggest.

It is recognized that the periods of testing the soils were so infrequent that many significant changes in the soil population may have been overlooked. The points of maximum effects and of inflection would undoubtedly have been more accurately located by more frequent observations of the soils. It is apparent from the data which are available, however, that certain pronounced differences are expressed in the abundance of the organisms at different stages of plant growth.

In view of the present observations it would seem that plant growth is one of the most important factors in determining the irregular distribution of microorganisms in soils. Samples of soil from an apparently uniform field may vary greatly in abundance of organisms if they are gathered near or away from roots or if they contain a small or large amount of roots. The influences of the plants vary so greatly during the course of root development that observations from a single period would not be representative of other periods. In this connection, variations in the influences of the plants upon the organisms at different stages of growth may be of importance in determining the so-called seasonal fluctuation of microörganisms in soil, where some of the variations appear to be related to neither the moisture nor temperature. At least, it may be concluded that samples of soil gathered about different plants at any one period give no true representation of the comparative influences of the different plants upon the soil population. Any comparison which is to represent the facts properly must indicate the course of the influences throughout the growth of the plants.

The fact that plant growth favors the appearance of larger numbers of organisms in the soil, at least at certain stages in plant development, indicates why, under certain conditions one may expect to find some close correlations between crop yields of soils and abundance of microörganisms in the soils. Provided that the soils concerned in the comparison were originally alike and that the same crop had been continuously planted on these soils, one would expect that, where the greatest plant development occurred, there would be the greatest amount of root residues and that the other effects associated with the influences upon the organisms would be such as to exert the most profound effects upon the soil population. On the other hand, knowing that different plants affect the soil population differently at any stage in their growth it would hardly be expected that even similar soils supporting different plants under different fertilizer treatments would always show correlations between yields of plants and abundance of microörganisms in the soils. This relationship is further complicated where soils essentially different in physical and chemical condition are concerned. Even though crop yield should be high from a soil which was normally of low biological activity the abundance of organisms might not be so great as from a soil naturally of a high level of biological activity. At the same time the actual increase in abundance of organisms resulting from plant development might be the same in both soils. The growth of crops in rotations further complicates an interpretation of the final results upon soil organisms.

SUMMARY

Results are reported of studies concerned with the influences of the development of higher plants upon the abundance of microörganisms in the soil. A considerable variety of different plants was cultivated in the greenhouse and field and, during their growth, observations were made periodically upon the

abundance of bacteria, actinomyces, filamentous fungi, organisms developing upon nitrogen-free mannite agar, organisms of the *B. radiobacter* group developing upon nitrogen-free mannite agar, organisms of the *B. radiobacter* group developing upon glycerol-nitrate agar, and nitrogen-fixing organisms developing in nitrogen-free dextrose or mannite solutions. The following conclusions appear to be justified as a result of these studies:

1. The development of higher plants exerts pronounced influences upon the soil population but these influences are greater upon some organisms than upon others. The proportional increases in nitrogen-fixing organisms, actinomyces, and filamentous fungi are slight. The greatest proportional increases appear in the *B. radiobacter* group of organisms although very striking effects are apparent in the general bacterial population. The average of the seasonal effects of different plants upon the different bacteria varied between increases of 15 per cent and several hundred per cent.

2. Different plants exert different degrees of influence upon the soil organisms; some, as potatoes, consistently increased the numbers slightly, while others, as rape, increased the numbers to a striking degree in most cases.

3. The extent of the influence of any one plant upon the soil population is different at different stages of growth. Slight effects are apparent in early stages of growth, maximum effects appear only after the plants have reached considerable size, and the influences become less pronounced subsequent to the death of the plants. Consequently, the length of the growing period is an important factor in determining the degree of the effect of plants upon microörganisms. Because of the longer growing periods of biennials these plants show a much more prolonged effect upon the organisms than do annuals. Legumes may exert no more pronounced effects upon soil organisms than do non-legumes.

4. The extent of the effects of plants upon the soil organisms is not determined by the size of the different plants or the extent of root development but may be associated with some characteristics of the physiology of the plants, particularly as regards quality and quantity of root excretions. Many factors are undoubtedly concerned in these changes.

5. The results emphasize the fact that higher plants are of great importance in bringing about an unequal distribution of microörganisms in the soil and may be a major factor in determining the so-called seasonal fluctuation of microörganisms in soil where temperature and moisture do not appear to be related.

REFERENCES

(1) Smith, N. R. 1928 The identification of *B. radiobacter* and its occurrence in soil. *Jour. Bact.* 15: 20–21.

(2) Starkey, R. L. 1929 Some influences of the development of higher plants upon the microörganisms in the soil: I. Historical and introductory. *Soil Sci.* 27: 319–334.

(3) Wilson, J. K., and Lyon, T. L. 1926 The growth of certain microörganisms in planted and in unplanted soil. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 103: 1–25.

THE BOTANICAL COMPOSITION AND MORPHOLOGICAL FEATURES OF "HIGHMOOR" PEAT PROFILES IN MAINE[1]

A. P. DACHNOWSKI-STOKES

Bureau of Chemistry and Soils, United States Department of Agriculture

Received for publication January 29, 1929

During recent years the study of peatlands and their differentiation has tended more and more to emphasize the importance of rather detailed and varied knowledge of profile features. It has come to be regarded a necessary part of any successful attempt in bringing peat soils into agricultural or industrial use, to approach the examination of peat profiles upon the basis of the botanical composition and morphological characteristics of the several layers and "horizons" found at different depths from the surface to the mineral subsoil. Likewise an increasing amount of attention is given the effort to characterize more completely any type of peat area with all its properties and peculiarities, that is, to determine accurately the several outstanding physical properties, the chemical composition of various organic complexes, and the microbiological population of peat profiles under virgin conditions, in order to contribute to an understanding of the changes that take place in similar areas of peat under conditions of cultivation and lowered water table.

Most of the criteria by which we determine the outward characteristics and geographical mode of origin of peatlands, and analyze their different profile features are relatively well known and readily applied. But the problem becomes involved and often very complex when we attempt to coördinate the results of the various analyses, or when we seek to establish the probable degrees or stages of decomposition from the initial, virgin peat profile to the fully developed mature profile, to arrange the changes in a proper sequence, to determine the rate of decomposition and time involved, as well as their relationship to any given environment in which the changes occur.

The result of focussing the efforts of modern peat investigations upon the examination and analysis of the profile features of three raised bogs or highmoors in Maine furnishes the theme for the following two related studies. This paper constitutes an endeavor to give the botanical and morphological profile record reflecting the succession of former vegetation units now forming peat deposits, whereas the paper by Waksman and Stevens, published elsewhere in this issue of the journal, emphasizes certain chemical and bacterio-

[1] A detailed analysis of the chemical composition and the bacteriological activities in the various layers of these profiles is published elsewhere in this issue of the journal, by Waksman and Stevens.

logical methods as the approach to the complex problem of establishing relationships between different types of peat (5). A discussion of the physical properties and the coördination of analytical studies with regional profile differences is contemplated in a later paper.

The field work was carried on in coöperation with S. A. Waksman from August 6 to August 15, 1928, and afforded not only the opportunity of continuing earlier studies on New England peat profiles (1) but also of obtaining fresh material, especially from layers of peat at considerable depth below the surface.

The localities chosen were the Denbo heath near Cherryfield, the Veazie heath southwest of Orono, and the Lewiston or Garcelon heath at Lewiston, Maine. Aside from the matter of accessibility these areas of peat are better known than others, and further experimental work can be carried on with the use of the facilities of the university and agricultural experiment station at Orono as a base.[2]

METHODS OF PROCEDURE

The study of the peat profiles, the botanical identification of the successive layers of plant remains, and the separate morphological features were carried out as follows:

Peat samples from the surface to 3 feet, intended for obtaining analytical data from the standpoint of volume weight and for determining the changes in certain physical properties, were collected by means of a new type of sampler. The instrument was made by C. J. Crawley in the mechanical laboratory of this Bureau. It consists of a duraluminum or brass tube approximately 1½ inches (3.75 cm.) in diameter and 10 inches (25 cm.) long. At one end is a detachable device 2 inches (5 cm.) long provided with a slit about 1¾ inches (4.5 cm.) from its cutting edge. The sampler is inserted without turning into the walls of the freshly dug hole. The peat material in the outer, projecting edge of the tube is cut off with a spatula through the slit-like opening in the tube. The surplus material from the inner, inserted end of the tube is cut off with a sharp knife even with the cutting edge of the tube. The undisturbed core of the peat sample, having a definite unit volume equal to approximately 3 cubic inches (5 $cm.^3$), is then dropped into a small "ointment" glass jar provided with a tightly fitting aluminum screw-cap. From a series of determinations on peat profiles of southern states it was found that the procedure lent itself to an accurate determination of the volume-weight of different layers of peat in a profile and was important in order to interpret properly various peat soil phenomena in connection with plant responses.

Samples of peat from the entire profile depth were obtained by the use of the American peat sampling instrument which is a modification of the device

[2] The writer wishes to express his appreciation to Dr. Steinmetz of the University of Maine for aid rendered in sounding the Veazie peat area.

first designed by the Wisconsin Geological Survey. The instruments made in the mechanical laboratory of this Bureau consist of a cylinder of duraluminum or of brass, approximately 10 to 14 inches (25 to 35 cm.) long and with an inside diameter of $\frac{3}{4}$ inches (2 cm.). The cylinder is provided with a plunger cone-shaped at the lower end, and with a spring-catch near the upper end. The spring permits the withdrawal of the plunger from its enclosing cylinder at any desired depth. When locked in that position the instrument may be filled with a solid core of the respective peat layer by a downward movement. The cylinder protects the peat sample completely from any contamination and does not destroy its structure when the instrument is lifted out. Beginning at the surface downward, samples of peat are taken consecutively with the greatest care from distances either at every 6 inches (15 cm.) or 1 foot (30 cm.) from each other. The samples are placed into sterilized containers, glass vials, or any other holder, suitably marked, by forcing the spring catch and the plunger back into the cylinder. A number of 2-foot (60-cm.) sections of $\frac{1}{2}$ inch (1.7 cm.) duraluminum or of steel pipe can be readily connected by couplings. The lengths permit an easy estimation of the depth reached from which the peat sample is required. The instrument is kept in a canvas case, carried by a strap over the shoulder, and is very easy to handle. Tools such as the Swedish chamber drill of the Hiller model, boring sticks, and augurs have not given satisfactory results.

For the correct determination of the plant remains which have contributed to the formation of different layers of peat, an ecological herbarium with emphasis upon the plant community as the vegetation unit, and a collection of representative types of peat material are indispensable. The different types of peat found in this country even at great distances apart show a striking correspondence with those which occur in Europe, and they appear to have been derived from units of vegetation which on the whole are ecologically related. The necessary condition for the comparison of sharply demarked differences between types of peat with corresponding series of peat materials in Europe is, of course, the botanical and ecological method. The system of classification described in Departmental Bulletin 802 and the photographic illustrations published in Departmental Bulletin 1419 or in von Post's recent paper (4) permit with a fair degree of certainty the identification of the more important types and mixtures of peat materials found over practically the whole of the United States, Canada, and Europe. However, a compilation of an illustrated handbook of plant remains, including microscopic forms in peat deposits, is greatly to be desired and should be undertaken by the aid of international coöperation. Thus the main basis for coördination in peat investigations—reference of different kinds of peatlayers to their common origin and composition, and to one and the same standard of quality—would be rendered less difficult.

Preparations for microscopical examination of plant remains and their transition stages are handled in the laboratory in the following manner: Fresh ma-

terial is washed with distilled water and the smaller component parts of organic material are separated in water to which a few drops of chloral hydrate have been added. Air-dry peat samples are boiled in a 10 per cent solution of potassium hydroxide, essentially according to the method of Erdtman (3). Fractions of the peat material are prepared as microscopic slides in distilled water to which a drop of glycerine is added. Pollen grains and other minute plant remains are determined more or less quantitatively on samples which represent a fairly uniform layer of peat. The studies in pollen statistics require, however, much more development because the pollens of various species of deciduous plants resemble one another closely. Degrees of decomposition, following von Post's scale of ten divisions, are indicated as being poorly (H0-2), slightly (H2-4), partly (H4-6), largely (H6-8), and well (H8-10) decomposed. It is obvious, however, that a more exact quantitative method must be established to express the degree of decomposition that has taken place at different levels.

PEAT PROFILE RECORDS

Lewiston (Garcelon) heath

The topographical and geographical position of this deposit is shown on the Lewiston sheet of the U. S. Geological Survey. The area is located near the eastern city limits of Lewiston, Me. It exhibits a convex surface contour rising more than 5 feet (1.5 m.) from margin to center. The borders had at one time a considerable growth of spruce and tamarack with birch and alder but much of the timber has been cleared off by cutting and repeated fires. The conifers of the boreal climax are being replaced by white pine, red maple, and a dense growth of deciduous shrubs. Root sprouts of *Populus tremuloides* are occasional. The evergreen heath consocies in the central part of the peat area is dominated by *Chamaedaphne calyculata*, averaging between $1\frac{3}{4}$ to $2\frac{3}{4}$ feet (53 to 83 cm.) but *Kalmia glauca* and *K. angustifolia* are giving the locality a distinctive aspect in many places. Minor elements are *Ledum groenlandicum*, *Andromeda polifolia*, *Aronia nigra*, as well as *Spiroea* and *Solidago*. The chief element in the ground-cover consists of Polytrichum mosses (*P. strictum*, *P. commune*) favored by fire, and several lichens (*Cladonia rangiferina*, *C. uncialis*, *C. sylvatica*), while low small cushions of sphagnum mosses, chiefly mixtures of *S. tenellum*, *S. acutifolium*, *S. fuscum*, and others, occur in protected places but much suppressed.

The area has a marginal drainage ditch and a portion of it was formerly exploited in an attempt to manufacture peat fuel briquets. The drainage factor does not appear to have brought about much change in the ericaceous heath shrub complex but the deposit shows evidences of having shrunk vertically and compacted.

The profile sounding taken in the western half of the heath, about midway between the wooded border and the center is as follows:

1. 0–4 inches (0–10 cm.) very dark brown, largely decomposed, crumbly peat from Sphagnum and Polytrichum mosses with an admixture of ericaceous woody fragments and fibrous roots and culms of cotton-grass(*Eriophorum* sp.) moderately moist.

4–16 inches (10–40.5 cm.) light reddish brown, poorly decomposed fibrous, matted, moist Sphagnum peat (*S. cuspidatum*, *S. tenellum*, etc.) with an interlacing network of rhizomes and roots of the living surface vegetation.

16–20 inches (40.5–50.8 cm.) dark grayish brown, partly decomposed Sphagnum moss peat with fibrous culms and roots of cotten grass (*Eriophorum* sp.), small amounts of ericaceous woody fragments and finely divided organic debris; moist, firm, on

20–30 inches (50.8–76.2 cm.) dark reddish brown, wet Sphagnum moss peat, partly fibrous with moderate amounts of woody material from decaying branches and roots of shrubs and occasional conifers.

30–72 inches (76.2–182 cm.) reddish brown Sphagnum moss peat, in large part *S. cuspidatum* and *S. fuscum*, wet, compacted, more or less layered, contains at various levels small amounts of coarsely fibrous culms of cotton grass and coniferous root and stem fragments.

2. 72–86 inches (182–218.5 cm.) dark brown partly disintegrated ericaceous woody material, moist, compact, with admixture of partly fibrous moss and sedge peat. Marginal portions of this layer contain well-preserved leaves and slender woody fragments from Chamaedaphne and other heath shrubs, over

86–96 inches (218.5–243 cm.) dark brown to reddish brown, wet, partly decomposed fibrous moss peat (*S. fuscum*, with interlacing roots of woody perennials; *S. cuspidatum* etc.) marsh gas given off abundantly.

3. 96–120 inches (243–304 cm.) reddish to grayish brown coarsely fibrous, matted reed (Phragmites) peat on finely fibrous radicellate sedge-reed peat representing a former floating mat; weak odor of H_2S.

4. 120–156 inches (304–400 cm.) grayish brown sedimentary organic suspension, firm, (Dy and Detritus-Gyttja) with rootlets of sedges and reeds, grading into an olive brown, finely divided, more or less colloidal organic debris (Plankton Gyttja) which contains seeds of herbaceous plants and pollen from conifers.

5. 156+ inches (400+ cm.) gray to bluish gray, soft, sticky clay, compact at lower level, reported to be glacial Lake Champlain clay.

The profile examination indicates that the primary layers consist of limnetic sediments from an ancient shallow lake which proably represents the remnant of a former valley lake of considerable size. Sedge and reed marches continued the accumulation as a result of a further fall of the water level, and a forest began to spread over the telmatic types of peat after the lake had dried up. The formation of sphagnum peat began at a later, moister, postglacial period, but at present sphagnum mosses no longer continue their growth and dominance in the area. The surface material resembles a dark colored muck-like organic debris, somewhat mineralized partly from fires, and exhibits a marked physical change from the parent peat layer.

Veazie heath

The location, acreage, and topography of this peat deposit are shown on both the Orono sheet of the U. S. Geological Survey and on the map which accompanies the 1909 U. S. Soil Survey report of the Orono area. The deposit appears to be unconnected with the more extensive area of peat at Pushaw lake. In surface contour it is moderately convex, rising only a few feet from the wooded margin to the open growth in the center.

The vegetation presents the aspect of scattered small islands of dwarfed spruce, tamarack, and birch as boreal dominants with the usual ericaceous heaths grouped around them. These islands grow directly upon sphagnum moss peat in which the long and ramifying roots are embedded a foot or two (30–60 cm.) below the surface. Between the islands are low hummocky masses of Sphagnaceae on which trailing and erect but small-sized evergreen shrubs are about equally distributed. In the hollows between the hummocks there is an open growth of two sedges, *Scirpus caespitosus* and *Eriophorum vaginatum*, the latter being more frequent in the wetter spots with *Sarracenia purpurea*, *Arethusa bulbosa*, and *Cypripedium* sp. The characteristic plants upon the bright red cushions of *Sphagnum tenellum* are *Vaccinium oxycoccus* and *Drosera rotundifolia*.

The greatly dwarfed heath shrubs are dominated by *Chamaedaphne calyculata*. The subdominants are *Kalmia*, *Ledum*, *Aronia*, *Betula*, and occasional clumps of *Gaylussaccia dumosa*. They rise out of a ground cover of mosses, chiefly mixtures of *S. fuscum*, *S. medium*, *S. acutifolium*, and *S. girgensohnii* with invading *Polytrichum* sp. in a sub-copious position.

Nearly all around the bog and along the highway where drainage conditions are improved spruce and larch are dominant, with birch, alder, and heath shrubs especially abundant. The trees are of ordinary size but in the marginal transition they become smaller, scattered, and on the central portion of the bog rarely exceeding a few feet in height.

Approximately 250 feet (75 m.) west of the Stillwater-Bangor highway, the following profile was recorded on a moderate rise in the open low, shrub cover:

1. 0–48 inches (0–120 cm.) light yellow-brown, fibrous, spongy matted, poorly decomposed, wet Sphagnum moss peat, chiefly from species found at the surface, slightly altered, grading into a somewhat darker colored material below the 10-inch level, with an open network of tough, slender roots, fibrous rhizomes, and stems of woody perennials. Marsh gas escaping from lower level.

48–60 inches (120–152 cm.) brown, partly fibrous Sphagnum-Eriophorum peat with an admixture of ericaceous coarse woody fragments, loosely matted, waterlogged.

60–96 inches (152–243 cm.) water pocket with brown, soft, more or less suspended organic debris (Detritus-Gyttja); firmer at lower level.

2. 96–136 inches (243–345 cm.) dark brown, partly decayed, woody peat from logs, branches, and roots of conifers, birch, and shrubby undergrowth, embedded in finely fragmented woody debris, grading into

3. 136–140 inches (345–355 cm.) grayish brown detritus with rootlets of woody plants and sedges, granular when dry, over clayey sedimentary organic debris (Clay-Gyttja) somewhat elastic, on

4. 140+ inches (355+ cm.) gray, compact, fine sand with rootlets of herbaceous and woody plants at upper level, mottled with light gray spots below.

This profile suggests that an open, grassy forest invaded the area and continued for some time the accumulation of woody peat. The transformation of the forested peat area into a sphagnum covered moss bog occurred probably

at a time of the rise and expansion of the water surface of neighboring lakes. Thereafter sphagnum continued its dominance and formed a more or less floating layer of light brown, loose, coarse textured peat, with no evidence of marked physical change below the present surface.

Denbo heath

The conspicuously dome-shaped convex surface which rises 15 to 20 feet (4.5 to 6 m.) from margin to center, and the small ponds encountered on the highest top-parts, form the outstanding features of this area of peat. It is located about 18 miles (28 Km.) northwest of Cherryfield and covers an acreage of several square miles.

Although on the whole, the surface vegetation is the same as that of the other areas described there are yet minor differences. The present heath consocies is dominated by *Chamaedaphne calyculata*. The sub-dominants are *Kalmia glauca*, *Ledum palustre*, *Andromeda polifolia*, and several species of *Vaccinium*. Minor elements are *Eriophorum vaginatum*, *Scirpus caespitosus*, *Rhynchospora alba*, *Aronia nigra*, *Gaylusssaccia dumosa*, *Solidago sp.*, and greatly dwarfed conifers scattered over the bog. The ericaceous heaths, which in the transitional forested margin may grow to 5 feet (1.5 m.) in height, average only 5 to 7 inches (12 to 17 cm.) on the raised part of the bog. The ground-cover shows abundant mats of different species of Sphagnum mosses with *Vaccinium oxycoccus*, *Drosera rotundifolia*, and *Sarracenia purpurea*, but Cladonia and Polytrichum in particular are suppressing them, indicating drier conditions.

About the small ponds occur nearly pure carpets of *Sphagnum tenellum*, *S. magellanicum*, *S. acutifolium*, with Scirpus and Eriophorum dominating in narrow transition belts. A further stage in the succession are the irregular-shaped islands of low, dwarfed spruce and tamarack, about 2 feet (60 cm.) tall, with a fringe of ericaceous heaths, showing a tendency to occupy the hollows that are made by knoll-like hummocks of *Sphagnum fuscum* and other mosses.

Fire has been occasional but there appears to be no tendency toward the establishment of a fire sere or subsere.

The vegetation which constitutes the more or less encircling forest is characteristically dominated by spruce, tamarack, and birch with an ericaceous undergrowth. In the transitional zone and on the rising slopes the trees and shrubs generally become smaller. The great dwarfing and scantiness of conifers distinguish the raised from the flat portions of the peat area.

The test boring nearly midway between the wooded margin and a pond on the dome-shaped surface gave the following profile record:

1. 0–5 inches (0–12 cm.) thin superficial layer of grayish brown, partly decomposed leafy litter and granular debris embedded in Sphagnum mosses on light yellow brown fibrous moss peat (derived mostly from living species), poorly decomposed, spongy matted, moist, with interlacing slender rootlets.

5–12 inches (12–30 cm.) reddish brown, partly decomposed Sphagnum peat (*S. recurvum?*,

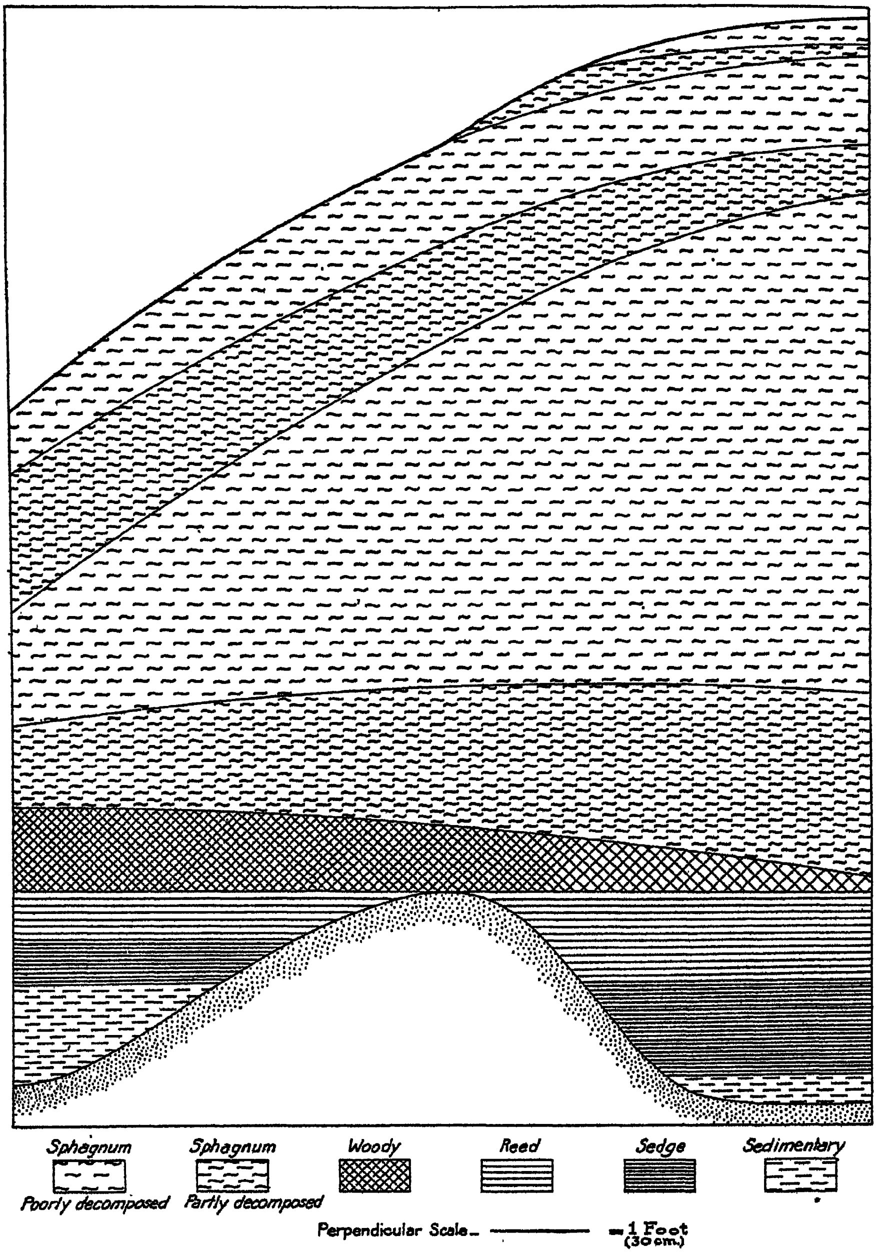


FIG. 1. CROSS SECTION OF A PORTION OF DENBO HEATH SHOWING MODE OF GROWTH AND DISTRIBUTION OF SUPERIMPOSED STRATA OF SPONGY FIBROUS MOSS PEAT WHICH HOLDS LARGE AMOUNTS OF WATER, ALTERNATING WITH MORE DECOMPOSED, COMPACT, AND DARKER COLORED STRATA OF SPHAGNUM MOSS PEAT

Compiled from series of profile soundings made between 1924 and 1928

S. imbricatum, and others), moist, somewhat firm, with embedded network of long tough woody stems and fibrous roots of *E. vaginatum* at lower level. Standing water fluctuating 10 inches (25 cm.) below surface.

12–28 inches (30–71 cm.) light reddish brown Sphagnum peat slightly decomposed, fibrous, loose, wet, with traces of course and fine rootlets of woody perennials, and occasional thin streaks of firmer, darker colored organic debris.

28–48 inches (71–121 cm.) brown, fairly compact Sphagnum peat, wet, in more advanced stage of decomposition than upper strata, with woody fragments of rather local distribution and abundance.

4–17 feet (121–518 cm.) light reddish brown to brown fibrous porous Sphagnum peat (includes mixtures of *S. fuscum*, *S. cuspidatum*, and others) poorly decomposed, wet, with open network of rootlets and occasional coniferous and ericaceous woody fragments, darker in color, firmer and partly decomposed below 13 foot (396 cm.) level; escape of marsh gas from various lower levels.

2. 17–18⅔ feet (518–568 cm.) brown woody peat from branches, twigs, and poorly decomposed coarse woody roots of heaths and conifers, embedded as a tough interlacing network in waterlogged, finely divided organic debris; difficult to penetrate. Pollen of alder, birch, and spruce intermingled.

3. 18⅔–22 feet (568–670 cm.) gray-brown, coarsely fibrous, matted reed (Phragmites) peat, containing ericaceous woody rootlets at upper level, forming a transition to radicellate sedge (Carex-Scirpus) peat, darker in color at lower level, firm, slightly moist, grading into

4. 22+ feet (670 + cm.) thin layer of brown to greenish brown sedimentary peat (Clay-Gyttja) with conifer pollen, on compact fine sand.

From a series of profile soundings which were made between 1924 and 1928 the cross section shown in figure 1 has been drawn to illustrate the various stages in the development of the dome-shaped convex form. It will be observed that darker colored, partly decomposed, compact, and relatively impervious moss substrata alternate with spongy, fibrous, poorly decomposed, light colored moss peat which holds considerable amounts of water in its capillary spaces. The whole layer of moss peat appears to have accumulated obviously under the influence of changes in atmospheric conditions and with distinct regularity: expanding masses which concentrated in the center, were followed by younger strata which diminished in extent with the growth in height. The transformation into a convex surface is on the whole, independent of any hollows, knolls, or ridges of the underlying sandy bottom. The features of the present surface vegetation point clearly to increasingly drier conditions, indicating that the peat no longer receives and absorbs a copious rainfall or condenses heavy fogs.

SUMMARY

The results presented in this paper bring out the following facts as to the essential characteristics of three peat profiles in Maine.

1. A consideration of the surface aspects shows light colored poorly to dark colored partly decomposed phases of Sphagnum moss peat varying in thickness from 5 to 17 feet (152 to 518 cm.), with a water table fluctuating between 3 to 10 inches (7.5 to 25 cm.) below the surface.

2. An examination of the internal composition and variation in profile structure indi-

cates that the layer of Sphagnum moss peat is superimposed upon a layer of woody peat moderately decomposed, followed in deeper depressions by a fibrous reed-sedge peat over sedimentary peat resting upon a clayey to sandy mineral substratum. Stratigraphically the peat profiles represent the conifer heath-sphagnum moss series and the sedimentary-reed sedge-conifer heath-sphagnum moss series.

3. The layers of peat are throughout under anaerobic conditions. The rate of decomposition is exceedingly slow at present, since the profiles show largely the inherent features of the component parent materials. In terms of stage of development (toward peat soil formation) the areas may be grouped into the category of immature, virgin peatlands, predominantly botanical in character.

4. Physiognomically the surface vegetation may be designated as shrubby heath-moor; it represents a successional stage passing into the conifer sub-climax.

5. The raised, dome-shaped surface is due chiefly to an upward mode of growth and periodic accumulation of Sphagnum mosses; it is probably related to a maritime climate (von Post's region of ombrogene peat deposits) which was until recently much moister than that now existing in the northeastern Atlantic coastal region. The partly decayed sub-layers indicate probably the influence of temporary dry periods (2).

6. The areas of peat appear to have come into existence during a relatively recent period in post-glacial times. The general agreement in stratigraphic features probably relates to a common age of the deposits, the Lewiston peat being relatively older than the Veazie or the Denbo peat.

7. The peat areas may be included into a major division characterized on the one hand by the lack or the removal of nutrient mineral salts (low ash content) in any layer of peat or horizon near the surface (oligotrophic group of peat lands), and by the presence, on the other hand, of conditions which give to the organic material an acid reaction.

REFERENCES

(1) Dachnowski, A. P. 1926 Profiles of peat deposits in New England. *Ecology* 7: 120–135.

(2) Dachnowski-Stokes, A. P. Records of climatic cycles in peat deposits. Carnegie Inst. Wash. In press.

(3) Erdtman, G. 1924 Studies in the micro-palaeontology of postglacial deposits. *Jour. Linn. Soc. (London)* Bot. 46: 449–504.

(4) Post, L. v. och, Granlund, E. 1926 Sŏdra Sveriges Torvtillargangar. Sveriges Geol. Undersŏkn. Årsbok. 19, no. 335, Ser. C.

(5) Waksman, S. A., and Stevens, K. R. 1928 Contribution to the chemical composition of peat: II. Chemical composition of various peat profiles. *Soil Sci.* 26: 239–251.

CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: IV. CHEMICAL STUDIES OF HIGHMOOR PEAT PROFILES FROM MAINE

SELMAN A. WAKSMAN AND KENNETH R. STEVENS

New Jersey Agricultural Experiment Station

Received for publication January 22, 1929

Peats are formed as a result of a certain one-sided decomposition of plant residues, in the presence of an excess of water, which makes conditions anaerobic and prevents the distinctly aerobic processes from taking place. However, if this were the only factor responsible for peat formation, the chemical composition of all types of peat would be alike. The fact that we have several types of peat which are distinct from one another in their chemical make-up points to other factors responsible for this difference. Among these, the nature of the vegetation and the environmental conditions under which decomposition is taking place, especially reaction and abundance of nutrients, are most important. Highmoor peats, lowmoor peats, forest peats, and sedimentary peats have certain characteristics in common, namely, the abundant accumulation of organic matter resistant to decomposition and a certain tendency toward a similarity in physical and chemical nature. But they are still distinctly different from one another, and can readily be recognized and well described, not only by their botanical composition as commonly assumed, but also by their chemical composition. A highmoor peat possesses certain definite characteristics, not merely because its plant associations are made up of various species of *Sphagnum*, *Eriophorum*, and various Ericaceous shrubs, but because these plants possess a definite chemical composition and because their chemical complexes will decompose only in a certain definite manner, especially at a high acidity and with a low mineral content, such as prevails in that type of bog. The lowmoor peat owes its specific composition not merely to the specific plants, but to the chemical composition of these plants and to the conditions under which they are undergoing decomposition. The same fact is true of the other types of peat.

In the decomposition of complex plant substances by microörganisms, the water-soluble substances are among the first to be attacked, and are followed soon by the pentosans and celluloses. The lignins are most resistant to decomposition, although it has been definitely established that at least some fungi belonging to the Hymenomycetes and possibly certain bacteria and actinomyces are capable of attacking lignins; the specific fungi may decompose these as rapidly as if not more so than they do the celluloses. However, this

process can take place only under aerobic conditions, whereas under anaerobic conditions, as in the case of the peat bogs, the lignins are hardly decomposed at all. Klason (9) many years ago was the first to express ideas of this nature when he stated that the processes whereby dead plants are changed into the first stages of peat must consist in the fermentation of carbohydrates; when this takes place in the absence of air, or when the organic matter is covered with water, the process must stop here, since the microörganisms active under these conditions are unable to ferment the other plant constituents; peat was thus considered to consist of lignin-like substances of plant origin in a more or less unchanged condition.

But the mere assumption that the formation of peat from plant residues consists in a gradual decomposition of the pentosans and other hemicelluloses as well as of the celluloses, and an accumulation of lignins or of lignin transformation products (so-called "humic acids") does not explain the marked differences in the chemical composition of the various peats as well as in the decomposition processes. Highmoor peat is characterized by a low ash content, by a high cellulose, fat, and wax content, by a fairly large amount of hemicellulose, and by a low protein content. The distinguishing characters of a lowmoor peat are the practical freedom from true celluloses, an abundance of proteins, a high mineral and lignin content, and a very low fat and wax content.

Analyses of various horizons of a highmoor peat profile from Germany were reported elsewhere (15). These results led to the conclusion that the formation of this type of peat is accompanied by a rapid decomposition of some of the nitrogenous complexes of the plant residues and a slow decomposition of the carbohydrates; these include not only the lignin-like complexes, but also the hemicelluloses and celluloses, as well as waxy substances. In the lowmoor peat formations, the reverse is true, namely, a gradual accumulation of the nitrogenous complexes and a rapid disappearance of the celluloses and hemicelluloses take place; the lignins accumulate but the ether-soluble substances do not.

Oden and Lindberg (12) reported that although Sphagnum peat contains 4.3 to 6.6 per cent ether-soluble substances and 3.3 to 4.7 per cent cellulose, Carex peat contains none at all or very little of either of these two groups of complexes. Hesz and Komarewsky (8) isolated 10 per cent cellulose from air-dry peat, presumably of the highmoor type; the same peat contained 6 per cent ether-soluble, 10 per cent alcohol-soluble, and 49 per cent material soluble in cold 1 per cent NaOH solution, including the pentosans, gums, pectins, etc.

Although Cajander (4) and others were largely justified in their assumption that chemical analyses of peat are worthless unless their botanical composition is well known, we possess also definite information that not only does the botanical composition of peat depend entirely upon the chemical conditions prevailing in the bog, especially the abundance of available nitrogen, calcium, and other minerals (16, 10), but that the two are closely interrelated.

Among the important factors which influence the vegetation in peat bogs, the absolute reaction occupies a prominent place. Olsen (13) found that when the pH of the bog is 4.2 or less, a true highmoor vegetation predominates, including *Eriophorum vaginatum*, *Oxycoccus*, *Calluna*, *and Andromeda*. With a decrease in pH value, the number of species diminishes. According to Brenner (3) typical sphagnum peat has a pH value of 3.9 to 4.5.

To throw further light upon the chemical composition and the nature of the organic complexes in the highmoor types of peat, three sphagnum bogs located in Maine were studied. A description of the location of these bogs, their botanical composition, as well as the profile features are published elsewhere in this issue of the journal by Dachnowski-Stokes.[1]

These three bogs were located as follows: 1. About eighteen miles from Cherryfield, Maine, and designated as *C*; this bog is known locally as the "Denbo heath" or "Deblois heath," near the town of Deblois; this is a typical highmoor bog rising in the center twenty to twenty-five feet from the outer margin; the bog has been described in detail by Bastin and Davis (1), Nichols (11), and Dachnowski (5).[2] 2. The second bog examined was located at the border of Pushaw Lake, a few miles southwest of Orono and also known locally as the "Veazie heath" or the "Orono bog" (designated as *O*). 3. The Garcelon bog (bog *L*) about two miles east of Lewiston, Maine, has been described by Bastin and Davis (1) and Dachnowski (5).

The following samples from these three bogs were used for chemical and bacteriological investigations:

Cherryfield bog:

C_1, Upper layer of the Cherryfield bog, at a depth of 2 to 8 cm., consisting largely of young *Sphagnum* peat with an admixture of some *Eriophorum* and various Ericaceous plants.

C_2, 8–20 cm. level, forming a darker layer, consisting also of *Sphagnum*, with an admixture of woody fragments from Ericaceous shrubs and *Eriophorum*.

C_3, 20–30 cm. level, reddish brown peat consisting of practically pure *Sphagnum*.

C_4, 30–46 cm. level, pure *Sphagnum* peat.

C_5, 46–61 cm. level, pure *Sphagnum* peat.

C_8, 183–214 cm. level, pure *Sphagnum* peat.

C_9, 460–480 cm. level, pure *Sphagnum* peat.

C_{10}, 550–580 cm. level, sedge peat, lowmoor formation.

Orono bog, about 330 cm. depth; no sedimentary layer at bottom; the bog resting upon forest formation. Surface vegetation consisting of *Sphagnum* (*Sph. squamosum*, *Sph. tenellum*), with an admixture of *Eriophorum*, *Saracena*, and various shrubs (*Ledum*, *Cassandra*, *Andromeda*, *Kalmia*, *Vaccinium*) as well as of tamaracks and spruces.

O_1, surface layer of peat, 1–10 cm. deep.

O_2, 15–20 cm. layer, light *Sphagnum* peat.

---

[1] The sampling and general examination of these three bogs were carried out together with Dr. Dachnowski-Stokes of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, to whom the authors are indebted for the helpful assistance rendered. The authors take this opportunity to express their indebtedness also to Dr. Steinmetz of the Maine Agricultural Experiment Station, who assisted in the location and sampling of the Orono bog.

[2] See also (7). Compare some of the analyses reported by Dachnowski (6).

O_3, 20–30 cm. layer, light *Sphagnum* peat.

O_4, 90–120 cm. layer, light *Sphagnum* peat.

O_5, 150–180 cm., *Eriophorum* and woody peat admixture.

O_6, 240–270 cm. layer.

O_7, 270–330 cm. layer, forest peat, bottom of deposit.

Lewiston bog found to be about 425 cm. deep, at the place of sampling, with a marked sedimentary layer at the bottom of the profile.

L_1, Surface 1–10 cm. level, consisting of *Polytrichum-Eriophorum-Sphagnum* peat.

L_2, 20–25 cm. level, *Sphagnum* peat.

L_3, 40–50 cm. level, *Sphagnum* peat.

L_4, 60–75 cm. level, *Sphagnum* peat.

L_5, 240–270 cm. level, sedge peat.

L_6, 360–400 cm. level, sedimentary peat, with a clay underground.

The moisture content of the original peat samples and the reaction, as expressed in terms of hydrogen-ion concentrations (determinations made by means of a quinhydrone electrode using Leeds and Northrup Type K potentiometer) are given in table 1. The samples were immediately air-dried as soon as brought to the laboratory and chemical analyses were made on the air-dry material. The ash and nitrogen content of the different samples are also given in table 1.

The results show that the typical sphagum peats are, in agreement with the results obtained by other investigators, highly acid in reaction, having a pH of 4.0 or even less. The *Sphagnum* layers are low in ash and in nitrogen. When the sedge peat is reached, there is an immediate rise in the pH value and in the ash and nitrogen content. In other words, the higher the acidity of the bog the less is its nitrogen and ash content. It is important to note that the minerals are usually more concentrated at the surface of the bog than in the lower levels, until the lowmoor or forest horizons are reached. Birk (2) also found previously that, although the ash content of the peat in the profile diminishes from the bottom toward the surface, there may be another rise near the very surface.

However, the three bogs used in these investigations show considerable differences in this connection, depending entirely upon their topography and vegetation.

The Cherryfield profile, which is the most typical highmoor peat, shows in the upper layers a variation in pH value from 3.73 to 4.05; there is a gradual decrease in acidity at a depth of 180 cm. reaching in the horizon of the sedge peat a pH value of 5.18. The ash content varies in the upper sphagnum layers from 0.90 to 1.14 and is only 2.00 per cent at the immediate surface, but there is a considerable increase in the sedge layer to 2.83. The nitrogen varies in the sphagnum layers from 0.653 to 0.839 per cent, but in the sedge layer the nitrogen has increased to 1.845 per cent.

In the Orono peat, the reaction is somewhat less acid, varying in the sphagnum peat horizons from pH 3.95 to 4.35, increasing rapidly to pH 5.70 in the sixth layer and to 6.04 in the lowest forest layer. The somewhat higher pH

value is accompanied by a higher ash content, ranging from 1.45 to 3.05 per cent in the sphagnum peat horizons, with an increase to 8.01 per cent in the sedge peat horizon and to 9.34 per cent in the lowest layer of the forest peat. The nitrogen content is just as low in the sphagnum horizons of this bog as in those of the previous bog (0.635 to 0.915), but there is a rapid increase in nitrogen in the woody-*Eriophorum* layer, even greater than in the sedge peat layer.

TABLE 1

Moisture content, reaction (pH), ash, and nitrogen content of various layers of three highmoor peat profiles

SAMPLE NUMBER	DEPTH OF LAYER	MOISTURE CONTENT OF ORIGINAL SAMPLE	REACTION OF FRESH PEAT	ASH CONTENT OF DRY MATERIAL	NITROGEN CONTENT OF DRY MATERIAL
	cm.	*per cent*	*pH*	*per cent*	*per cent*
C_1	1–8	92.7	4.05	2.00	0.653
C_2	8–20	92.6	3.95	1.14	0.684
C_3	20–30	92.6	3.85	1.02	0.817
C_4	30–46	92.9	3.86	0.90	0.839
C_5	46–61	93.6	3.73	1.06	0.770
C_8	183–214	93.4	4.47	0.99	0.705
C_9	460–480	92.4	4.71	1.10	0.756
C_{10}	550–580	92.2	5.18	2.83	1.845
O_1	1–10	94.2	4.35	3.05	0.635
O_2	15–20	93.9	4.30	2.80	0.646
O_3	20–30	91.8	3.95	1.84	0.915
O_4	90–120	93.7	4.13	1.45	0.885
O_5	150–180	95.0	4.20	1.74	2.105
O_6	240–270	92.6	5.70	8.01	2.151
O_7	270–330	89.9	6.04	9.34	1.931
L_1	1–10	88.3	4.10	8.79	1.135
L_2	20–25	93.6	3.99	1.85	0.683
L_3	40–50	92.8	3.98	1.68	0.924
L_4	60–75	90.0	4.15	1.25	1.240
L_5	210–240	90.1	6.28	4.24	.....
L_6	360–400	92.1	6.86	23.58	2.911

The Lewiston peat shows again somewhat different relationships due to the difference in the nature of the plant associations that have contributed to the make-up of this bog: horizons 2 to 4, which are typical sphagnum layers, have a pH value of 3.98 to 4.15, an ash content of 1.25 to 1.85, and a nitrogen content of 0.640 to 1.240; the very surface layer of the peat is rich in ash (8.79 per cent) and in nitrogen (1.135 per cent), but it is also fairly acid (pH 4.10). There is a rapid decrease in acidity in the sedge horizon and especially in the sedimentary peat, where it reaches nearly the neutral point; a parallel increase

is observed in the ash content. The nitrogen content of the peat also increases with depth, especially in the sedimentary peat.

The chemical analyses of the various horizons of these three peat profiles have been carried out according to the methods outlined previously (14). The results are reported in tables 2 to 4.

The chemical complexes found in the eight different horizons of the Cherryfield bog (table 2), taken at various depths from the surface to a depth of 20 feet, show all the characteristics of a highmoor peat. This is brought out by the large amount of fats and waxes (ether-soluble fraction), by the high cellulose and hemicellulose content, and by the low protein and ash content. The ether-soluble and alcohol-soluble substances increase with depth, the hemicelluloses and celluloses decrease, and the lignin-like complexes increase. It is interesting to note that the change from the sphagnum peat to the sedge peat in the deepest sample taken (C_{10}), which is characterized by an

TABLE 2

Chemical composition of a highmoor peat profile located near Cherryfield, Maine

On per cent basis of dry material

HORIZON NUMBER	ETHER SOLUBLE	ALCOHOL SOLUBLE	HEMI-CELLULOSES	CELLULOSES	LIGNINS	PROTEINS	ASH	TOTAL
C_1	2.35	1.45	26.45	16.86	27.18	4.08	2.00	80.37
C_2	2.62	1.92	25.24	14.74	29.21	4.28	1.14	78.95
C_3	2.82	1.83	24.55	15.97	28.85	5.11	1.02	80.15
C_4	2.57	2.08	22.25	13.69	32.23	5.24	0.90	78.96
C_6	2.96	3.20	18.48	14.66	33.24	4.81	1.06	78.41
C_8	3.97	3.15	15.94	15.55	37.43	4.41	0.99	81.44
C_9	4.89	4.29	12.69	11.85	44.83	4.73	1.10	84.38
C_{10}	5.97	5.06	5.96	5.06	54.11	11.53	2.83	90.52

increase in pH value, in the nitrogen and in the ash content, shows also a considerable drop in the abundance of celluloses and hemicelluloses and a rapid increase in the lignin content. These phenomena are characteristic of lowmoor peat formations in typical lowmoor profiles. When the chemical composition of this highmoor profile from the extreme northeastern portion of the United States is compared with the composition of the highmoor peat profile obtained in Germany, as recorded elsewhere (15), it is found that the two are very much alike.

In other words, independent of the region, topography, and history of the bog, the chemical composition of a definite layer in a peat profile depends entirely upon the nature and chemical composition of the vegetation from which this layer originated and the conditions under which its decomposition has been carried out.

Just as a thin layer of sphagnum peat found in a forest peat profile (15) had all the chemical characteristics of various layers of sphagnum peat in typical

highmoor profiles, so does a layer of sedge, of forest, or of sedimentary peat in a highmoor peat profile possess all the chemical characteristics of similar layers in lowmoor peat profiles or sedimentary peats. In other words, these results show conclusively that peats, considered not only from the point of view of the profile as a whole but also as a series of different layers varying in origin and composition, can be characterized by their chemical composition; this may have even a more important bearing upon the applications to be made of these peat formations, than a knowledge of their botanical composition alone. To be able to characterize a peat both by its botanical and chemical composition is of course of even greater significance for gaining an understanding of bog formations and their practical utilization.

TABLE 3

Chemical composition of a sphagnum peat profile near Orono, Maine

On per cent basis of dry material

HORIZON NUMBER	ETHER SOLUBLE	ALCOHOL SOLUBLE	HEMI-CELLULOSES	CELLULOSES	LIGNINS	PROTEINS	ASH	TOTAL
O_1	1.76	1.40	26 30	16.43	19.15	3.97	3.05	72.06
O_2	2.53	1.39	25.51	13.33	22.23	4.04	2.80	71.83
O_3	2.45	1.90	20.92	16.23	25.43	5.72	1.84	74.49
O_4	2.57	2.20	22.68	12.07	25.83	5.53	1.45	72.33
O_5	3.63	2.83	15.78	10.84	35.75	13.15	1.74	85.01
O_6	2.60	2.16	5.93	3.20	52.79	13.44	8.01	88.13
O_7	2.73	2.06	4.78	2.70	54.94	12.07	9.34	88.62

TABLE 4

Chemical composition of a sphagnum peat profile at Lewiston, Maine

On per cent basis of dry material

HORIZON NUMBER	ETHER SOLUBLE	ALCOHOL SOLUBLE	HEMI-CELLULOSES	CELLULOSES	LIGNINS	PROTEINS	ASH	TOTAL
L_1	2.90	2.10	18.09	9.72	26.96	7.09	8.79	75.65
L_2	1.73	1.37	26.57	14.06	21.49	4.27	1.85	71.34
L_3	4.83	2.75	13.82	11.40	35.85	5.78	1.68	76.11
L_4	3.55	3.81	11.15	9.08	47.47	7.75	1.25	84.06
L_6	2.24	1.86	5.60	1.40	37.44	18.19	23.58	90.31

Table 3 gives the analyses of the Orono peat profile. In this bog as well, the upper four horizons consist largely of sphagnum peat which is characterized by a high acidity, a low ash and nitrogen content, a high hemicellulose and cellulose content, and a rather low lignin content. The fifth horizon (O_5) forms a transition to the sedge layers of peat. This is characterized by a slow but gradual increase in pH value, a rapid increase in the nitrogen, a gradual but marked decrease in the celluloses and hemicelluloses, and a definite increase in the lignin-like complexes.

The lowest two horizons of this profile consist of sedge and forest peat. In chemical composition, these two layers are found to be typical of sedge and forest peats. This is brought out by a rapid decrease in acidity or increase in pH value, a decided increase in the ash content, a high nitrogen content, a decrease in the celluloses and hemicelluloses, and an increase in the lignins. A definite parallelism is thus found again between the acidity of the peat formation and the cellulose and hemicellulose content, while an inverse relation is found to exist between these and the high ash, protein, and lignin content. A high acidity (low pH) indicates a characteristic growth of *Sphagnum* and *Eriophorum*; it indicates the presence of certain types of celluloses and hemicelluloses and their slow decomposition; it indicates a low protein content due to insufficient activities of the microörganisms. On the other hand, a low acidity points to a vegetation of grasses, to a rapid decomposition of the hemicelluloses and the celluloses, and to an accumulation of the lignins, proteins, and minerals.

An examination of the results obtained in the analysis of the various horizons of the Lewiston peat profile tends to confirm fully the above considerations. Here as well, the upper four horizons are markedly acid in reaction and represent a peat formed by a mixed vegetation of *Sphagnum* and Ericaceous shrubs. In chemical composition, these horizons are very similar to the upper corresponding horizons of the Orono profile, with certain distinctive differences due of course to the fact that both the vegetation and the conditions of decomposition are somewhat different in the two bogs. The sphagnum horizons are highly acid, have a low ash (except the surface layer) and nitrogen content, a high cellulose and hemicellulose content, and a low lignin content; the lowest or the fourth of these horizons (L_4), still acid in reaction and low in ash, shows already a marked diminution in the hemicellulose and cellulose content and an increase in lignins. When the sedge and sedimentary horizons are reached, there is a decrease in acidity, an increase in ash and nitrogen content, a decrease in celluloses and hemicelluloses, and an increase in the lignins.

This third profile is not a typical highmoor as the Cherryfield profile is and as the German peat profile described previously was. It represents a certain intermediary stage in the nature of its vegetation; it does not show as distinct differences in the chemical composition as do the typical highmoor and lowmoor profiles. However, the chemical composition of the various horizons in even such an uncharacteristic profile throws definite light upon the nature of peat formations.

Attention is here again called to the fact that in the lowmoor and forest peats, as well as in the sedge, forest, and sedimentary layers of highmoor peats, the sum total of the various organic and inorganic complexes accounted for in the proximate chemical analysis is about 90 per cent and frequently more. However, in the sphagnum peats the sum total of the chemical constituents accounted for by this method is only 72 to 80 per cent. This is largely because

in the treatment of the peat with 2 per cent hot hydrochloric acid, following the treatment with ether, alcohol, and water, about 50 to 55 per cent of the organic constituents are brought into solution; but of this, only about a half is accounted for in the form of reducing sugars and recorded as hemicelluloses (after multiplying the reducing sugar by 0.9). Since the nitrogenous complexes and the ash fraction brought into solution by the dilute acid do not in account for more than 2 to 4 per cent of this extract, 20 to 25 per cent of the organic constituents of the sphagnum peat which are soluble in hot dilute acids, but which do not give any reducing sugars, remain unaccounted for. This fraction includes various pectins and gum-like complexes, which give on hydrolysis organic acids and other non-reducing substances. It may also include certain polysaccharides which give furfural even on boiling with dilute acid and which are lost therefore from the determination. A study of the chemical nature of this complex will soon be undertaken.

A large number of anaerobic bacteria were found in the various horizons of the profile. These bacteria are largely anaerobic in nature although many of them are facultative anaerobes and can, therefore, live also under aerobic conditions. These organisms were found in an active state and their numbers do not diminish with the depth of the bog but even tend to increase. They bring about the decomposition processes taking place slowly in the bogs. A detailed study of the nature of these organisms and their activities will be published in a subsequent contribution.

SUMMARY

A study has been made of the chemical composition of the organic constituents of three sphagnum peat bogs in Maine, one of which was a typical highmoor bog.

The sphagnum horizons are very acid in reaction, of a pH around 4.0. The high acidity is always accompanied by a low ash (except sometimes at the very surface of the bog) and nitrogen content; a high cellulose, hemicellulose, fat, and wax content; and a low lignin content.

With the transition of the sphagnum layers into a sedge, forest, or sedimentary peat layer, there is an immediate rise in the pH value, an increase in the ash, a decrease in the cellulose and hemicellulose, and an increase in the protein and the lignin contents.

The chemical composition of the organic and inorganic complexes of peat bogs is quite sufficient for the description of these bogs, and a knowledge of this composition is of special importance from the point of view of the practical utilization of the bogs. When this information can be combined with a careful botanical description of the vegetation which has contributed to the formation of the various horizons, the results are of even greater significance for our understanding of the nature of the peat formations, their origin, and the methods of handling when brought under cultivation.

REFERENCES

(1) Bastin, E. S., and Davis, C. A. 1909 Peat deposits in Maine. U. S. Geol. Survey Bul. 376.

(2) Birk, C. 1914 Das tote Moor am Steinhuder Meer. *Arb. Lab. Tech. Moor. verm. K. Tech. Kochsch. Hannover.* 1: 1–102.

(3) Brenner, W. 1924 Über die Reaktion finnländischer Böden. Agrogeol. Julkais, Helsinki. No. 19.

(4) Cajander, A. K. 1913 Studien über die Moore Finnlands. Acta Forest. Fennica. 2.

(5) Dachnowski, A. P. 1926 Profiles of peat deposits in New England. *Ecology* 7: 120–135.

(6) Dachnowski, A. P. 1924 The chemical analysis of various peat materials by means of foodstuff analyses. *Jour. Agr. Res.* 29: 69–83.

(7) Ganong, W. F. 1897 Upon raised peat bogs in province of New Brunswick. *Trans. Roy. Soc. Canada* 3 (Sect. IV): 131–163.

(8) Hesz, K., and Komarowsky, W. 1928 Ueber Isolierung und Nachweis von Cellulose in Torf. *Anorg. Chem.* 41: 541.

(9) Klason 1893 Cited by Oden and Lindberg (12).

(10) Kotilainen, M. J. 1928 Untersuchungen über die Beziehungen zwischen der Pflanzendecke der Moore und der Beschaffenheit, besonders der Torfbodens. Wiss. Veröffentl. Finnisch. Moorkulturver. 7.

(11) Nichols, G. E. 1919 Raised bogs in Eastern Maine. *Geogr. Rev.* 7: 159–167.

(12) Oden, S., and Lindberg, S. 1926 Einige Torfanalysen im Lichte neuzeitlicher Theorien der Kohlebildung. *Brennstoff-Chem.* 7: 165–170.

(13) Olsen, C. 1921 Studier over Jordbundens Brintionkoncentration og dens Betyduing for Vegetationen, saerlig for Plantefordelningen i Naturen. Meddel. Carlsberg Labor. 15: no. 1.

(14) Waksman, S. A., and Stevens, K. R. 1928 Contribution to the chemical composition of peat: I. Chemical nature of organic complexes in peat and methods of analysis. *Soil Sci.* 26: 113–137.

(15) Waksman, S. A., and Stevens, K. R. 1928 Contribution to the chemical composition of peat: II. Chemical composition of various peat profiles. *Soil Sci.* 26: 239–251.

(16) Waren, H. 1924 Untersuchungen über die botanische Entwicklung der Moore mit Berücksichtigung der chemischen Zusammensetzung des Torfes. Finska Moorkulturför. No. 5.

THE EFFECT OF SWEET CLOVER AND ALFALFA ROOTS AND TOPS ON THE FUNGOUS FLORA OF THE SOIL

THOMAS L. MARTIN

Brigham Young University

Received for publication January 26, 1929

Investigations on the influence of specific kinds of materials on the development of soil fungi have been rather limited. Some phases of the subject of soil mycology and of the part that soil fungi play in the economy of the soil are only in the beginning stage. Enough work has been done, however, to indicate that soils changed by the addition of various materials which influence bacterial content and activity, also influence changes in soil fungi (5). Waksman (6) quotes the results of some investigators in which it was found that the addition of stable manure stimulated the development of *Penicillia* and *Mucorales*, and that pure cellulose in the presence of available nitrogen brings about an extensive development of *Trichoderma*, *Fusarium*, *Verticillium*, *Monosporium*, and certain *Penicillia*. The writer (3) found *Mucor*, *Rhizopus*, and *Alternaria* predominating in alfalfa root treated soils, *Cladosporium* in straw treated soils, and *Aspergillus* and *Penicillium* in sweet clover root treated soils.

The previous work seems to indicate that different kinds of organic materials influence to some extent the types of soil fungi which are most active in the soil. In previous studies by the author, sweet clover and alfalfa tops and roots seemed to differ in substance enough to warrant further study, so the experiment described below was planned and carried through.

PLAN OF THE EXPERIMENT

One-gallon glazed jars were filled with a clay loam soil of pH 7.5. The soil used was secured at a depth of 6 feet in the spring of 1928, and was left exposed until September, 1928. No plant material had obtained a foothold. Very slight summer showers were all the moisture which the soil had received. The surface 2 inches of a pile of this soil was removed and the soil used for the experiment was secured from this depth down to 12 to 18 inches. One per cent of air-dried ground alfalfa roots having a total nitrogen content of 1.33 per cent was added to each of three jars. Similar jars of soil were treated with corresponding amounts of dried and ground alfalfa tops, sweet clover tops, and sweet clover roots having a nitrogen content of 2.25 per cent, 2.30 per cent, and 0.77 per cent respectively. Three jars of soil were used as controls. The alfalfa roots were obtained to a depth of 12 inches from a 5-year-old alfalfa field and the tops were taken from the first crop at about the half mature stage.

The sweet clover roots were obtained to a depth of 12 inches from a second-year growth of sweet clover and the tops were approximately one-third grown. The moisture content of the soils was then brought to 25 per cent dry basis. All of the jars were kept under laboratory conditions at room temperatures varying from 19° to 21°C.

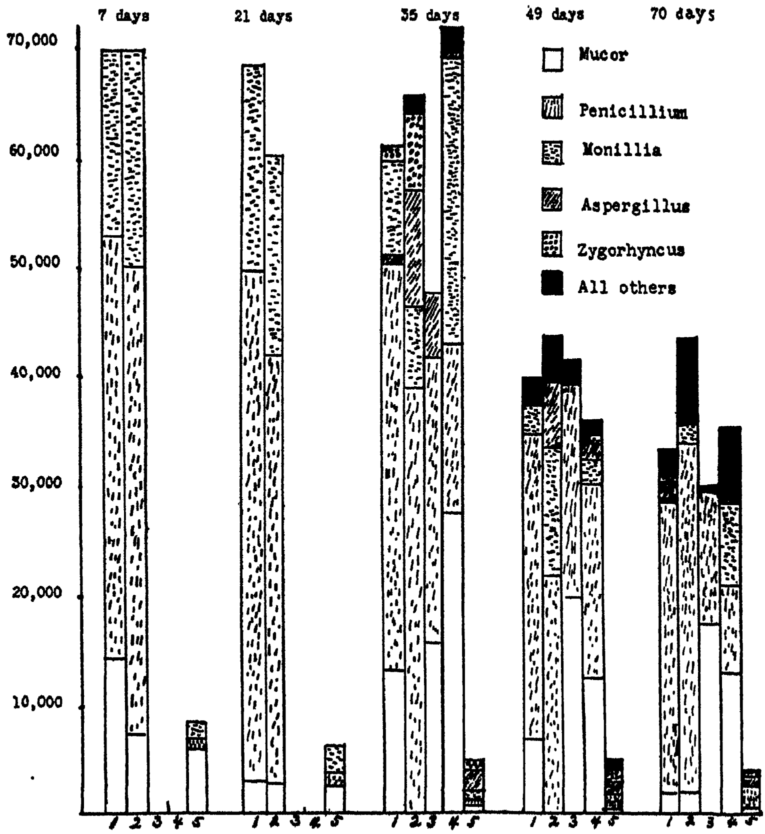


Fig. 1. Graph Indicating the Relative Development of Fungi at Different Periods of Time

Columns 1, 2, 3, 4, and 5 represent sweet clover roots, alfalfa roots, sweet clover tops, alfalfa tops and checks, respectively.

At intervals of 1, 3, 5, 7, and 10 weeks, the soils were sampled and mold counts and identification of mold genera were made for each sample. As previously mentioned, each soil was treated in triplicate. Four plates were poured from each sample, thus making 12 plates for each treatment. The average of each 12-plate group is given in the tables. Raisin agar described by Waksman (4) was used as the medium. The identification of the molds

produced on the plates was made according to the descriptions given by Buchanan (1), Gilman and Abbot (2), and Waksman (7).

DISCUSSION

It will be noticed that no counts are given in table 1 for the 7- and 21-day periods. When the plates were examined at the end of 3 days, they were so completely overgrown with species of *Mucor* that it was impossible to make counts. At the end of the 35-day period, the *Mucors* grew more slowly and the mycelium filled the plates less densely. It was, therefore, possible to make counts and study the types developed. The Penicillium colonies were very small. The results obtained at the 49-day period indicated a further retardation in the development of *Mucors*. There was, however, a slight increase in numbers. There was also a greater variation in the types developed. The 70-day period showed a very slow growth of *Mucors*. The Penicillium colonies were larger but fewer in number. The total number of molds for each period of determination diminished as the time of incubation increased.

As indicated in table 2, the sweet clover roots had a different influence on mold development. During the first period, the *Mucors* developed in larger numbers but their growth was very slow compared to the sweet clover top treatments. There were many large colonies of Monillia at first, but after 35 days, they decreased rapidly. The *Penicillium* colonies were the predominant fungi for clover roots in every period. Their numbers tended to decrease with time, but not so rapidly as did the Monillia and the *Mucors*. For some reason, the *Mucors* did not show much development during the 21-day period, particularly when compared with the 35-day period. The Monillia and Penicillia were about the same as in the previous sampling. In 35 days, the variation in types was more marked but all the colonies were very small. In the 49-day determination, it was found that the numbers had diminished, the *Mucors* grew more slowly and the Monillia were relatively quite large. The 70-day period failed to show much change in rate of growth and size of colonies. There were fewer fungi but the decrease occurred in all types, although the Penicillium showed the least relative change. There was also a slightly greater variation in mold type. As the incubation period lengthened the total number of colonies decreased in numbers, the same was noted for the tops, but the tops maintained its *Mucor* growth while the roots declined. Another significant difference is that the clover tops supported almost no Monillia, but the roots showed at least some in every period. The flora of the clover tops is largely confined to the *Mucor* and Penicillium types and is not nearly so variable as the tops.

Table 3 shows that alfalfa tops had about the same influence as sweet clover tops on *Mucor* development for the first 21 days of incubation. It was impossible to make counts because of the vigorous and rapid growth of the *Mucors*. The 35-day period indicates a predominance of *Mucors*, but they grew more slowly and did not fill the plates with mycelium. Monillia were

TABLE 1

Effect of sweet clover tops incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	....	....	48,000	43,000	32,812
Zygorhynchus	Plates overgrown with *Mucor* in 3 days		......	280	......
Monillia			......	1,200	312
Aspergillus			6,000	270	......
Penicillium			23,000	20,000	12,500
Mucor			19,000	21,250	20,000

TABLE 2

Effect of sweet clover roots incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	70,000	68,000	64,400	43,150	48,300
Sporotrichum	......	......	......	......	2,500
Botrytis	......	......	200	3,250	......
Zygorhynchus	......	......	1,250	......	1,000
Monillia	17,500	18,000	7,500	3,625	200
Aspergillus	......	......	400	......	1,500
Penicillium	37,500	47,500	37,500	29,000	26,250
Mucor	15,000	2,500	14,500	7,250	2,750

TABLE 3

Effect of alfalfa tops incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	....	....	72,450	36,780	38,650
Botrytis	Plates overgrown with *Mucor* in 3 days		......	......	200
Alternaria			......	......	8,200
Trichoderma			......	1,250	......
Rhizopus			4,000	......	......
Aspergillus			200	2,500	500
Monillia			25,000	2,500	6,500
Penicillium			16,250	16,250	7,500
Mucor			27,000	14,280	15,750

very noticeable in their development. All the other colony types were small; particularly was this the case with the Penicillium colonies which ranked third in number of colonies. At the end of the 49-day period, Rhizopus developed as also did the Trichoderma, although in total numbers, they did not approach the kinds mentioned above. The *Mucors* grew more slowly. At the end of 70 days, the *Mucors* continued to diminish in vigor, although not in numbers.

TABLE 4

Effect of alfalfa roots incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	70,000	61,000	60,500	47,000	17,800
Botrytis	......	......	......	3,500	......
Trichoderma	......	......	......	1,250	550
Alternaria	......	......	300	300	......
Zygorhynchus	......	......	6,500	7,500	......
Rhizopus	......	......	1,115	300	8,750
Aspergillus	......	......	11,750	......	......
Monillia	20,000	18,000	7,750	11,750	2,500
Penicillium	42,500	40,500	39,200	22,500	32,500
Mucor	7,500	2,500	......	......	2,500

TABLE 5

Numbers and types of soil fungi developed in soil without treatment with organic matter and used as controls for this experiment

Number per gram of soil and averages of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	7,900	7,550	4,055	4,740	3,200
Trichoderma	......	......	......	600	......
Zygorhynchus	......	......	1,125	1,000	200
Aspergillus	......	......	2,000	1,040	800
Rhizopus	......	......	......	300	......
Monillia	1,250	3,700	330	800	......
Penicillium	400	1,250	400	800	2,000
Mucor	6,250	2,600	200	200	200

The other types grew fairly large. There was a decrease in numbers as the length of time of incubation increased. This decrease was due to a diminution in all three of the main types of fungi but especially in the Monillia.

Table 4 presents the results following alfalfa root incorporations. The *Mucors* during the first period were few in number and their growth was very slow. The Penicillia grew quite rapidly and the colonies were relatively large

compared to the previous organic matter treatments. The Monillia grew more vigorously than in any of the previous treatments, with the possible exception of alfalfa tops. The 21-day samples indicated a very similar growth to the first week, the *Mucors* being fewer and slower in growth. The 35-day determinations showed a greater variation in colony types. There was no *Mucor* growth. The Monillia colonies were the largest that had been noticed thus far in the investigation. The 49-day period, as in the previous one, failed to show any *Mucor* development. There was a greater variation in types and the Monillia were the predominating colonies on the plates. The 70-day studies indicated a growth very similar to the 49-day determinations. The numbers had decreased more than previously.

The results from the controls, given in table 5, show a very poor growth of molds. The colonies were very slow in growing and the numbers were very few compared to the treated soils. During the 7-day period, the *Mucors* were small and the Monillia large. In three weeks the colony development had diminished. In five weeks there was a greater variation in types but fewer numbers. This was indicated also in the 7-week and 10-week determinations.

The most noticeable fact in all of these studies is that sweet clover tops and alfalfa tops stimulated a very vigorous *Mucor* development during the early part of the incubation period. The roots encouraged a greater variation in types. The Monillia colonies developed very noticeably in the alfalfa-treated soils, particularly with the root incorporations.

Comparing the number of colonies found in the two sources of organic matter, it is significant to note that the *Mucors* do not grow so abundantly in alfalfa as in clover. The only exception to this is for the tops during the 35-day period and the roots during the second period, when they were equal. The Monillia, on the other hand, are distinctly more numerous in the alfalfa treated soils, especially for the tops, and for the roots during the latter part of the experiment. The Penicillia do not show any well-defined reaction to either source of plant material, but are irregular at the different times when counts were made. On the whole, alfalfa tends to support a good growth of a wider variety of fungi than does clover, but during the first two periods when the greatest numbers of organisms were at work, the roots of both supported no recognizable fungi except *Mucor*, Penicillia, and Monillia.

Organic matter is a factor in mold development. One year ago, the writer attempted a similar study using a soil that had been cultivated for several years and had a nitrate content at the beginning of 40 p.p.m. The variation in types of organic materials added failed to show differences in mold development. The soil used in this experiment, however, never had grown a crop and the nitrate content at the beginning was 13 p.p.m. To study the influence of different kinds of organic matter on mold development, it is evident that a soil low in organic matter is essential. It may be that the nitrogen content of the organic material added is a distinct factor in the type of mold

development. The rate at which this nitrogen is made available also must be considered because in the soils when a vigorous nitrate accumulation occurred there was the greatest *Mucor* development.

CONCLUSION

When a clay loam subsoil is treated with alfalfa roots and tops and sweet clover roots and tops, these treatments influence the types of molds developed on that soil. The more succulent the material, the greater the stimulation to the development of *Mucors*. As the period of decay lengthens, there is a noticeable decrease in total numbers and also in the rapidity of development, particularly of the *Mucors*. The alfalfa root and sweet clover root incorporations do not influence the development as much as do the corresponding tops, but the roots increase the number of Penicillia more than do the tops.

Mucors grow more rapidly in soil containing either the tops or the roots of sweet clover than they do with incorporations of the corresponding parts of alfalfa, but the opposite may be said of the Monillia. The incorporation of alfalfa in a soil favors a wider variety of molds than does the incorporation of sweet clover. In general, the total number of colonies is greater in alfalfa treated soil than in sweet clover treated soil. After a period of 35 days, or after the subsidence of the vigorous growth of *Mucors* following the incorporation of the tops, the total number of colonies of molds is greater in soils treated with roots than it is in soils treated with tops of either alfalfa or sweet clover, and this is largely due to the greater number of Penicillia, although the roots also tend to have a greater variety of molds.

REFERENCES

(1) Buchanan, R. E. 1926 Bacteriology, p. 511–547. New York.
(2) Gilman, J. C., and Abbott, E. V. 1927 A summary of soil fungi. *Iowa State Col. Jour. Sci.* 1: 225–345.
(3) Martin, T. L. 1927 Decomposition studies of alfalfa and sweet clover roots and straw. *Soil Sci.* 24: 309–315.
(4) Waksman, S. A. 1922 The growth of fungi in the soil. *Soil Sci.* 14: p. 153–157.
(5) Waksman, S. A. 1926 Principles of Soil Microbiology, p. 238–239. Baltimore, Md.
(6) Waksman, S. A. 1926 Principles of Soil Microbiology, p. 260. Baltimore, Md.
(7) Waksman, S. A. 1926 Principles of Soil Microbiology, p. 246–257. Baltimore, Md.

THE GRAVIMETRIC METHOD FOR THE DETERMINATION OF CARBONATES IN SOIL

NORMAN ASHWELL CLARK AND EMERSON R. COLLINS

Iowa State College[1]

Received for publication January 28, 1929

Of the three methods generally used for the determination of carbonates in the soil—titrimetric, volumetric, and gravimetric—the gravimetric has fallen to a certain extent into disfavor and has received little attention for some time. Cain (5) cited many of the difficulties encountered in the absorption of carbon dioxide and the weighing of the absorbent, and soil chemists have not made any serious effort to improve the process, although it is generally accepted that the method gives very accurate results if precautions are observed (16).

The procedure can be stated briefly as the production of carbon dioxide from the carbonates by the action of dilute acid on the soil; the purification, drying, and absorption of the resulting gas, by drawing it through a "train" by aspiration; and the final weighing of the absorbing material. The chief precautions needed are: 1. the preliminary sweeping out of all traces of carbon dioxide from the apparatus; 2. the decomposition of all the carbonates in the soil without an attacking of the organic matter—a difficulty shared with the gas-volumetric and titrimetric methods; 3. the removal of the air from the absorption bulb with the same water content as it has on entering. Errors may also be incurred by the variation in the amount of moisture on the surface of the absorbing bulb. In the apparatus described hereafter, we have made an adaptation which shortens the time needed for sweeping out the carbon dioxide; the decomposition of the carbonates has been accelerated; the time taken both for the absorption and for the weighing has been materially reduced.

The old method of heating the soil with strong acid in order to set free the carbon dioxide was abandoned because of the action on the organic matter. Marr (10) at Rothamsted, after trying a variety of methods, finally distilled the soil with dilute HCl under reduced pressure at 50°C. for 20 minutes. He claimed that under these conditions no decomposition of organic matter took place, and carbonates were readily decomposed. Marr at the same time confirmed the conclusion of Amos (2) that occlusion of carbon dioxide in air-dry soil did not take place to any extent; this is also in accordance with our observations. MacIntire and Willis (8) suggested H_3PO_4 for the acid, but re-

[1] Contribution from the Department of Chemistry,

turned later to HCl (9) as the phosphoric acid failed to give complete decomposition of all carbonates in 30 minutes. 1:10 HCl was recommended by them for most soils and 1:5 for soils with greater than 15 per cent $CaCO_3$. They state "We have found that the action of 1:10 HCl on soil organic matter at room temperature is altogether negligible upon 5, 10, 20 gm. charges of the average soil." They also observe that if residual magnesite occurs in a soil "neither boiling for 1 minute, or several minutes, nor the Marr method, nor the Tennessee Station method will effect the complete decomposition of the mineral magnesium carbonate." Such soils are of rare occurrence, but they needed boiling for 30 minutes to get complete decomposition.

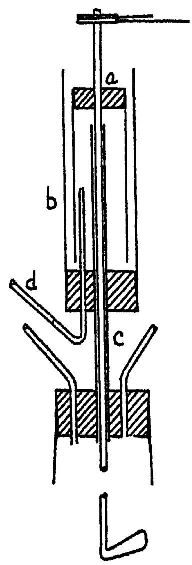


FIG. 1. STIRRER WITH MERCURY SEAL

MacIntire and Willis noted the necessity for shaking the soil with the acid and devised a multiple shaking apparatus which was later adopted by the Association of Official Agricultural Chemists. Truog (17) avoided the shaking by bubbling the CO_2-free air through the soil solution. In a previous report (6) we have noted the fineness to which soil is brought by continuous stirring, and in place of shaking we therefore introduced a stirrer combined with the mercury seal.

The stirrer with mercury seal was described by Brühl (4) and has become increasingly useful to the organic chemist (1). It can be applied frequently to soil problems. Figure 1 shows the stirring rod attached by a stopper (*a*) to the inverted tube which revolves with it. This tube is sealed to the passage of gas by the mercury in the outer tube (*b*). The tube (*c*) connects the seal

with the flask; the stirring rod, slightly greased, fits fairly closely into this. It is convenient to attach one of the small 1/8 H.P. stirring motors directly to the stirring rod to avoid vibration. An innovation is the presence of the extra inlet (*d*) which passes inside the revolving tube and terminates above the level of the mercury; this enables air to be drawn through the seal, effectively preventing any carbon dioxide from staying in the air spaces.

Preliminary stirring is unnecessary: a soil which was stirred with water free from carbon dioxide for 5 hours before adding the HCl, did not give any increase in the final amount of carbon dioxide compared to the same soil when stirred only during the time of aspiration. The speed of evolution of the gas without preliminary stirring was equal to the rate of production from the soil which had been previously stirred, indicating that the stirring during aspiration allows the acid to attack the carbonates with rapidity, as well as holding to a minimum the solution of carbon dioxide in the liquid (8). We have used a concentration of acid sufficient to make a 1:10 HCl in the flask when added to the soil and water. For soils with a high concentration of carbonates, or for lime materials, we have included sufficient acid to obtain approximately 1:10 HCl in the flask after the reaction, and have added the acid slowly.

A solution of potassium hydroxide is effective when used as the absorbent for the carbon dioxide but it necessitates slow aspiration. In order to accelerate the passage of the air, ascarite has been utilized. This sodium hydroxide, asbestos mixture was introduced in work on steel by Stetser and Norton (15) who found that the ascarite retained the water produced by the absorption of the carbon dioxide. Marsh (11), however, reported against its use for soils. He states that gases must be drawn or forced through the absorbing medium for periods varying from 12 to 48 hours or more, and as the ascarite lost water after 5 hours, he concluded that "where long runs are necessary as in the determination of CO_2 production from soil, ascarite because of loss of moisture, can not be successfully used."

A possible means of adapting ascarite for soils was pointed out by Underwood (18). In the direct determination of carbon dioxide in limestone Underwood passed the gas over phosphorus pentoxide before it entered a Midvale absorption bulb containing the ascarite, and again over the pentoxide before it left the bulb. In this way he took care of any excess moisture which was not absorbed by the ascarite. We have used a U tube with glass stopcocks for the absorbing materials. Approximately one-half inch of phosphorus pentoxide is placed on glass wool on the ascarite where the gases emerge from the tube. This allows of much faster aspiration than if a potassium hydroxide solution is used, and it is easy to see when the material should be renewed.

A drying agent, which compares well with phosphorus pentoxide in absorptive capacity and has several advantages, is magnesium perchlorate trihydrate, sold under the name "dehydrite." This was tested by Willard and Smith (19) and has been used by a number of investigators for carbon determinations

(3, 14); it has been applied by Lee and Brown (7) to biological processes. The dehydrite can be used until it increases in weight 20 to 25 per cent, and does not become sticky when charging the tubes. It gives little resistance to the gas and when used shrinks so that it does not stick or clog. This can replace the phosphorus pentoxide, but we have used a somewhat larger quantity of the dehvdrite in the weighing tube.

In order to facilitate the removal of the tube from the train, the arms may be ground to make glass joints, instead of being attached with rubber connec-

TABLE 1

Weight of U tubes without aspiration

	MINUTES IN BALANCE CASE		
	5	15	25
Wiped with alcohol, etc.	82.8876	82.8877	82.8880
Repeated	82.8876	82.8879	82.8880
Repeated	82.8876	82.8876	82.8881
Wiped with alcohol, etc.	82.8879	82.8881	82.8882
Repeated	82.8878	82.8881	82.8882
Wiped with alcohol, etc.	34.8027	34.8028	34.8029
Repeated	34.8026	34.8028	34.8029
Repeated	34.8026	34.8028	34.8029

TABLE 2

Weight of U tubes with aspiration

TREATMENT	HOURS ASPIRATED	MINUTES IN BALANCE CASE			
		5	10	15	20
Preliminary	1.25	60.2356	60.2358	60.2360	60.2360
HCl added	1.25	60.2683	60.2682	60.2682	60.2683
	1.5	60.2685	60.2685	60.2688	60.2688
	4.5	60.2696	60.2695	60.2696	60.2696
	1.0	60.2696	60.2698	60.2699	60.2699
$CaCO_3$ *per cent*		0.515	0.515	0.514	0.514

tions. The U tube is wiped off with an alcohol-dampened cloth and afterwards with a dry cloth: it is then placed in the balance case for a few minutes before weighing. We have found, even with a large U tube weighing 80 gm. when filled, that the original weight rarely changes more than 0.2 to 0.3 mgm. when it is left in the laboratory air for the five or six hours necessary for aspiration. Tubes weighing from 40 to 60 gm. when packed with the ascarite and P_2O_5 or dehydrite give a volume of sufficient size for a large number of

soil determinations; weighing with a tare is generally unnecessary if tubes are wiped off and weighed at the beginning of the run, although the weight will alter on long standing. Table 1 gives figures for both large and small tubes after being wiped with alcohol. Weights were standardized and weighings made as outlined by Richards (13) and are reported to the nearest 0.1 mgm.

From the figures given it will be seen that the gain in weight is very slow after 5 minutes in the balance case. Table 1 shows that consistent results can be obtained by wiping with the alcohol-dampened cloth, followed by a dry cloth and weighing after 5 minutes. Pregl (12) suggests the final wiping should be with chamois skin in microanalytical work in order to prevent electrification, and this procedure can be recommended. Table 2 gives figures for an actual series of weighings during a run on soil, with the CO_2 calculated as $CaCO_3$. A sample of Iceland Spar gave the following results: weight after

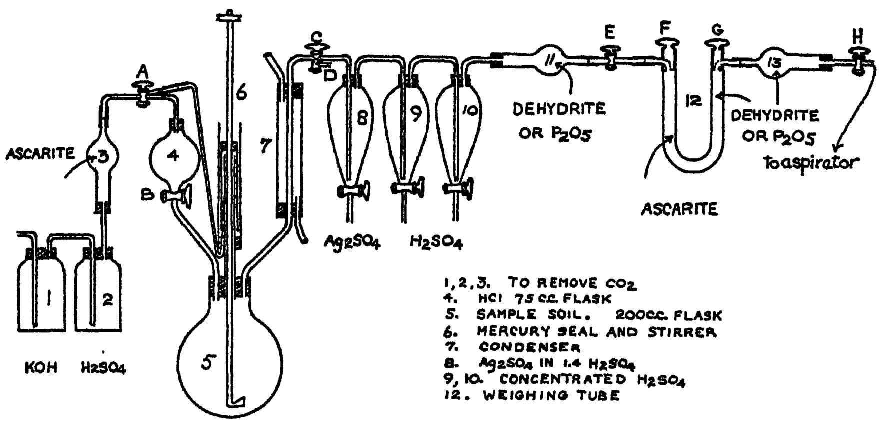


FIG. 2. APPARATUS FOR CARBON DIOXIDE DETERMINATION IN SOILS

5 minutes, 99.85 per cent $CaCO_3$; after 10 minutes, 99.84; 15 minutes, 99.85; 20 minutes, 99.86.

In figure 2, numbers 1, 2, and 3 serve to remove carbon dioxide from the air. The dropping funnel 4 is fitted with a rubber stopper, and is filled completely with the hydrochloric acid. A small water condenser, made of two glass tubes, has been found satisfactory for substances which do not need boiling; this protects from too much dilution the silver sulfate which is to catch the hydrochloric acid. When a silver precipitate forms it is easily removed through the stopcock at the bottom, and by creating a slight vacuum, a fresh solution is sucked up. The concentrated sulfuric in the following funnels can be replaced in the same way. The glass tubes in these are drawn to fine points, as in numbers 1 and 2, to decrease the size of the bubbles. Under the conditions the phosphorus pentoxide or dehydrite in 11 lasts for a long time without renewal. If the absorption tube 12 is fastened with rubber connections instead of ground glass joints, care must be taken that small

particles of rubber do not get inside the arms. Other joints may be wiped with benzene saturated with equal parts of paraffin and beeswax.

PROCEDURE

From 5 to 20 gm. of soil which has been passed through a 1-mm. sieve, is placed in flask 5 and washed down with a little CO_2-free water. Air is then drawn through the apparatus by running water from the aspirator; by the use of the three-way stopcock at *A* the CO_2-free air can be taken through the

TABLE 3

Carbon dioxide from soil, calcium carbonate and mixtures

Sample	Temperature	Weight of soil	Weight of $CaCO_3$	Aspiration to constant weight		Weight of CO_2	$CaCO_3$ in sample	$CaCO_3$ in soil
		gm.	gm.	hours	liters	gm.	per cent	per cent†
Iceland spar	Room	....	1.8171	5	15	0.7978	99.85	.....
Iceand spar	Room	....	1.9912	4	13	0.8748	99.92	.....
Iceland spar	Room	....	1.2210	4.5	14	0.5373	100.00	.....
Iceland spar	Room	....	1.9414	3.75	14	0.8534	99.98	.....
					Average		99.94	
Soil 10	Room	5.0000	......	5.25	14	0.1305	5.94	.....
Soil + spar	Room	5.0000	1.6219	4.5	19	0.8431	......	5.93
Soil 10	50°	5.0000	......	3.25*	9	0.1318	6.00	.....
Soil 22c	Room	10.0000	......	4	13.5	0.4898	11.14	.....
Soil 22c + spar	Room	10.0000	1.2430	6	17	1.0357	......	11.13
Soil 22c	50°	10.0000	......	4*	12	0.4942	11.24	.....
Soil 22c + spar	50°	10.0000	1.2430	1.25*	5	1.0373	......	11.17
$CaCO_3$	Room	....	0.3276	5.5	24	0.1398	97.05	.....
Soil 32	Room	15.0000	......	7.25	20	0.0340	0.52	.....
Soil 32 + $CaCO_3$	Room	15.0000	0.2757	5.75	32	0.1511	......	0.51
Soil 32	50°	15.0000	......	7.25*	16	0.0356	0.54	.....
Soil 32 + $CaCO_3$	50°	15.0000	0.2757	3.00*	14	0.1520	......	0.53

* After aspiration at room temperature as above. Final figures were the same when soil was run at 50° from the time of adding acid.

† Total in mixture minus amount in $CaCO_3$ as found at room temperature.

stirrer or through the funnel 4. If there are no leaks, when the inlet at 1 is closed for a few minutes, the water will cease to flow. Stopcocks are then closed and tube 12 is weighed as described, and the aspiration repeated until the weight is constant. The tube is replaced in the train and funnel 4 filled with the dilute hydrochloric acid. Before opening *B* stirring and aspiration are started, and then *A* and *B* turned to allow a few drops at a time to pass into flask 5. When all the acid is in the flask, aspiration proceeds slowly at first, and is gradually increased and continued until no change in weight in

the absorption bulb occurs. The CO_2-free air is drawn through the mercury seal or through funnel 4 as desired. Between runs the stopcocks are closed, so that the drying parts of the system 8, 9, 10, and 11 are kept free from the outside air. After the introduction of the soil into 5, the three-way stopcock may be opened at *D* and air drawn through from 1 to *D*; this materially accelerates the preliminary removal of the CO_2 from the apparatus.

The three soils reported contain very widely differing amounts of carbonate. It will be seen from table 3 that in the mixture of soil and carbonate, when the CO_2 in the Iceland spar is subtracted from the mixture, the recovery of the CO_2 from the soil is almost identically the same as it is when the soil only is used. At 50° there is a slight increase in the amount of CO_2 given off. MacIntire and Willis ascribe this to a decomposition of the organic matter at the higher temperature (8), and this seems to be the case. Saturated CO_2 water with HCl was aspirated and all the CO_2 was removed in about 2 hours at room temperature. No increase in weight was found by raising the temperature to 50°. Similarly the Iceland spar showed no increase in the weight of CO_2 on running at 50°. The indication is that the increase when soil is present is due to the action on the soil and not to any CO_2 dissolved in the water.

During the time of aspiration little or no attention is needed —five to seven hours has been found long enough in almost all cases for the U tube to come to constant weight.

SUMMARY

1. An improved method for the gravimetric determination of soil carbonates has been outlined.

2. The method makes use of ascarite and either dehydrite or phosphorus pentoxide as absorbents.

3. The soil mixture is stirred instead of shaken, and the mercury seal is adapted for this purpose.

4. One to ten hydrochloric acid is used at room temperature. At 50° there is a slight increase in the amount of CO_2 given off.

5. Figures are given for determinations on three soils containing widely differing amounts of carbonates, both alone and mixed with calcium carbonate.

REFERENCES

(1) Adams, R. et al. 1921 Organic Synthesis. New York.

(2) Amos, A. 1905 A method for the determination of carbonates in soils. *Jour. Agr. Sci.* 1: 322–326.

(3) Avery, S. 1928 Carbon and hydrogen determinations using a metal tube. *Indus. and Engin. Chem.* 20: 1232–1244.

(4) Brühl, J. W. 1904 Ueber einen Schüttel- und Rühr-Apparat. *Ber. Deut. Chem. Gesell.* 37: 918–924.

(5) Cain, J. R. 1914 Determination of carbon in steel and iron by the barium carbonate titration method. *Jour. Indus. and Engin. Chem.* 6: 465–468.

(6) Clark, N. A., and Collins, E. R. 1927 The quinhydrone electrode and the soil reaction. *Soil Sci.* 24: 453–463.

(7) Lee, M. O., and Brown, J. B. 1927 A note on the use of magnesium perchlorate trihydrate (dehydrite) and asbestos-sodium hydroxide (ascarite) in gravimetric metabolism determinations. *Jour. Biol. Chem.* 73: 69–72.

(8) MacIntire, W. H., and Willis, L. G. 1913 Soil Carbonates. A new method of determination. Tenn. Agr. Exp. Sta. Bul. 100.

(9) MacIntire, W. H., and Willis, L. G. 1915 The determination of soil carbonates. A modification. *Jour. Indus. and Engin. Chem.* 7: 227–228.

(10) Marr, F. S. 1908 Estimation of calcium carbonate in soils. *Jour. Agr. Sci.* 3: 155–160.

(11) Marsh, F. W. 1925 A test of ascarite, a carbon dioxide absorbent as its own drier. *Jour. Assoc. Off. Agr. Chem.* 8: 442–444.

(12) Pregl, F. 1917 Die Quantitative Organische Mikroanalyse, p. 38–39. Berlin.

(13) Richards, T. 1900 Note on a method of standardizing weights. *Jour. Amer. Chem. Soc.* 22: 144–149.

(14) Smith, G. F., Brown, M., and Ross, J. F. 1924 Magnesium perchlorate trihydrate—its use as a drying agent for steel and organic combustion analysis. *Indus. and Engin. Chem.* 16: 20–22.

(15) Stetser, J. B., and Norton, R. H. 1918 Combustion train for carbon determination. *Iron Age* 102: 443–445.

(16) Truog, E. 1915 Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titration method. *Jour. Indus. and Engin. Chem.* 7: 1045–1048.

(17) Truog, E. 1916 A new apparatus for the determination of soil carbonates and new methods for the determination of soil acidity. *Jour. Indus. and Engin. Chem.* 8: 341–345.

(18) Underwood, J. E. 1926 Apparatus for the direct determination of carbon dioxide. *Indus and Engin. Chem.* 18: 1069–1070.

(19) Willard, H. H., and Smith, G. F. 1922 The preparation and properties of magnesium perchlorate and its use as a drying agent. *Jour. Amer. Chem. Soc.* 44: 2255–2259.

THIS NUMBER COMPLETES VOLUME XXVII

Volume XXVII June, 1929 Number 6

SOIL SCIENCE

Contents

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
New Brunswick, N. J.

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to Dr. Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

SOIL SCIENCE is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Volumes begin with the January and July issues, respectively. The subscription price is $10.00 for Volumes XXV (Nos. 1–6 inclusive) and XXVI (Nos. 1–6 inclusive) January, 1928, to December, 1928, inclusive, United States, Mexico, Cuba. An additional charge per volume of 50 cents for subscriptions in other countries. *Back volumes* are supplied on orders for Vols. I to XIV inclusive, XIX to XXIV incl.—$100.00 per set.

New subscriptions are accepted to begin with the current issue, unless the subscriber gives instructions to begin the subscription with some other issue. If the subscriber desire, back issues of the current volume may be obtained when in stock. The publishers do not guarantee to supply back issues, however.

Renewal subscriptions. To avoid a break in your series, subscriptions should be renewed promptly on expiration. Otherwise it may prove impossible for the publishers to furnish the back issues missed, as only sufficient copies are printed of each issue to supply orders in hand.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; or G. Jervis Manton, 430 Lit. Collins St., Melbourne (and at Sydney and Brisbane)

For the British Empire, except Australia and Canada: Bailliere, Tindall & Cox; 8 Henrietta Street, Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann, Krausenstrasse 38, Berlin N. W., Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

Publishers of: *Medicine, Journal of Urology, Bulletin of the Johns Hopkins Hospital, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Physical Therapeutics, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thompson Research Laboratory, Journal of Biological Chemistry, Chemical Reviews. Journal of Dairy Science, Soil Science. Social Forces, Personnel Journal, Journal of Comparative Psychology. Comparative Psychology Monographs, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, American Speech, Childhood Education.*

ERRATA

American soils as seen by Russian investigators, by J. S. Joffe and I. Antipov-Karataev, Soil Science, Vol. XXVII

Papes 161—Second line from bottom, *240* should read *740*.
Page 162—Sixth line from bottom, *310* should read *31°*.

Microbiological activities in the soil of an upland bog in eastern North Carolina, by I. V. Shunk, Soil Science, Vol. XXVII

Pages 288 and 291, the cuts for figures 1 and 2 should be reversed, the legends being left unchanged in their present positions.

THE RELATIONSHIP OF SOIL TYPE TO THE CALCIUM AND MAGNESIUM CONTENT OF GREEN BEAN STEMS AND LEAVES AND OF THEIR EXPRESSED JUICE[1]

J. F. FONDER[2]

Michigan State College

Received for publication January 2, 1929

In a previous paper (9) the influence of soil type upon the calcium and magnesium content of alfalfa was discussed. Data on the relationship of soil type to the calcium and magnesium content of the bean plant are set forth in this paper.

Since beans grow readily under greenhouse conditions, thus permitting of carefully controlled experiments, it was possible not only to determine variations in the composition of the plants with respect to calcium and magnesium but also to study the relationships between certain characteristics of the soils and the calcium and magnesium content of the plants. These data are made a major part of the discussion included herein.

REVIEW OF LITERATURE

Burd (4) showed that the correlation between the amounts of plant-food in uncropped soil and the crop-producing power of the soil is sufficiently great to justify the belief that the amounts are a measure of the soil's crop-producing power. Duley (7) found no very definite correlation between the calcium in the soil solution and the soil's ability to produce crops under field condition. According to Russell (22), Brazeale's work shows that small variations in the concentration of the soil solution are without effect on the growth of plants, but Rothamsted's experiments have shown the growth of crops to vary directly with the concentration of the soil solution.

Since the addition of some fertilizers enriches the soil solution, as explained by Kelley (15), and since several workers (1, 10, 16, 17, 19) have found that the addition of fertilizers influences the composition of plants, it is possible that there is a relationship between the composition of the soil solution and the composition of plants.

Burd (4) asserts that good, uncropped soils may contain considerably more solutes than poor, uncropped soils, but both good and poor soils are reduced to the same low level by growing plants. Hoagland (13) states that from

[1] Part 2 of a thesis presented to the graduate committee of Michigan State College in partial fulfillment of the requirements for a degree of doctor of philosophy.

[2] The writer wishes to express sincere appreciation to Dr. M. M. McCool for his kindly suggestions and criticisms in the execution and presentation of this work.

neither the water extract nor the freezing point method is there any indication that the soil solution has a constant composition; on the other hand, the soil solution may vary greatly under different conditions and a growing crop markedly diminishes its concentration. The soil solution under conditions favorable to crop growth was found by Hoagland (13) to be very dilute, especially during the height of the growing season. Duley (7) found the calcium content of Colby silt loam to be reduced from 22 to 54 per cent by a crop of clover. Stewart (23) showed that soils under crop always contain smaller amounts of calcium and magnesium than their uncropped duplicates. According to Burd (5), the water extracts of soils on which barley was growing showed variations in the calcium and magnesium content which were related to the content of these in the plants; as there was an increase in the plants, there was a decrease in the water extract. McCool and Millar (18) found that the freezing point lowering of the tops of plants was not very sensitive to changes in the concentration of the soil solution.

Duley (7) concluded there was a closer correlation between the amount of calcium in a soil soluble in 0.04 normal carbonated water and the soil's response to liming than there was between the pH value of the soil and this response.

Fleetwood (8) determined that the amount of calcium soluble in 0.04 normal carbonated water in the 0-7 inch layer of soil was closely related to the returns from application of lime to the soil. Swanson, et al, (24) showed a close correlation between the calcium content of the soil soluble in cold normal hydrochloric acid and the pH value of the soil, provided the soils were all of the same texture. Duley (7) ascertained that in nearly all cases the addition of lime increased the amount of calcium in the soil solution.

Russell (23) states that soils kept in dry conditions increase the amounts of their available plant-foods. Duley (7) found that very acid Kolby silt loam increased the concentration of the calcium in its soil solution when kept in the greenhouse for a number of months.

PLAN OF THE EXPERIMENT

Although primarily intended to show variations which may occur in the calcium and magnesium contents of green bean stems and leaves and in their expressed juice at different stages of growth, when the bean plants are grown on different soil types, the work presented here was planned also to show the effect of crop growth upon the concentration of calcium and magnesium in the soil solution and upon the pH value of the soil and the relationship of these to the composition of the plants. The textures of the soils in relation to the calcium and magnesium contents of the plants were also observed.

The experimental work consisted of growing Robust field beans in 2-gallon crocks containing surface soils of seven different types. Five pots of each soil type were planted with beans. One pot of each soil type was planted to grow 20 bean plants for the first sampling and the remaining four pots to carry 7 plants for the subsequent samplings.

The soil types used in this experiment included Roselawn loamy sand, Plainfield loamy sand, Kewanee sandy loam, Onaway sandy loam (heavy phase), Hillsdale sandy loam, Brookston loam (heavy), and Miami silt loam. The Roselawn was much more alkaline than is typical and the Plainfield had been limed. The Miami was very heavy and appeared to include at least some subsoil material. From the textural standpoint, the soils fell into two distinct groups, the one very light and including the sandy soils, and the other very heavy, consisting of the Brookston and Miami soils.

The analysis was planned to show the amounts of calcium and magnesium present in both the leaves and the stems and likewise in the expressed juice of each of these. The green weights of the stems and leaves were also obtained. The hydrogen-ion concentration of the soils and the amounts of calcium and magnesium present in the soil solutions were determined at each sampling. The soil solution consisted of the displaced liquid portion of the soil.

EXPERIMENTAL PROCEDURE

The experiments were begun in the greenhouse on February 2, 1928, when 35 two-gallon pots were planted to Robust field beans.

The first samples were taken on February 21, as soon as the plants were large enough to furnish enough material for analysis and the second when the plants were 6 weeks old. The third samples were obtained when flower buds had appeared, regardless of the age of the plants; the fourth, when the fruit was setting; and the fifth, when the pods were well filled out but the plant material had not started to wilt. All samples were taken between 8 and 10 a.m. and only on clear days, in order to reduce to the minimum variations due to shading and intensity of transpiration. As the plants were cut from each pot, the leaves and stems were separated, weighed immediately, and placed in a saturated atmosphere to prevent loss of moisture.

In the laboratory the plant parts were cut into very small pieces to allow a more representative sample to be taken. In most of the work, two or three grams of the green material was weighed out for total analysis and the juice extracted from the remainder.

The juice of the green material was obtained by the pressure method described by McCool and Weldon, (19) and at seven tons pressure per square inch. This great pressure was necessary because of the small amount of material available. The expressed juice thus obtained was not centrifuged but was weighed directly into crucibles and ashed along with the green material in a muffle furnace at dull red heat. Especially in the early stages of growth and with the expressed juice, the amount of each sample was so small that some method was required which would give the maximum amount of material for each determination. Therefore, the ashed material was taken up in 5 normal hydrochloric acid and made up to 110 cc. Of this solution, 50-cc. aliquots were used for each determination, allowing that five-elevenths of all the material be done in duplication.

The calcium determinations were made by the official volumetric method and the magnesium by the volumetric method of Handy (11). Twentieth normal $KMmO_4$ and KOH standard solutions respectively were used in each determination and the averages of only closely agreeing duplicates are reported here.

Immediately following the removal of the plants from the pots in the greenhouse, the soil was carefully sieved and portions were taken for the determination of the moisture content, the hydrogen-ion concentration, and the soil solution respectively.

The moisture content of the soil was based upon the weight of the absolutely dry soil obtained by heating in an electric oven at 105°C. for 15 hours.

The hydrogen-ion concentration was determined by the quinhydrone method of Briilman as described by Baver (2).

The suggestions as to technique made by Briilman and Tovborg-Jenson (3) and by Baver (2) were observed.

The soil solutions were obtained by the modified Lipman's direct water pressure method described by Burd and Martin (6) and supported by a number of workers, including Parker (2), Parker and Tidmore (21), and Hubbard (12). One thousand grams of soil was packed into the tubes, covered with 200 cc. of water and the soil solution displaced under pressure, which ranged from 40 to 50 pounds per square inch. The first 10 cc. of soil solution were discarded and the next 30 cc. caught and analyzed by the same method as was used for the first material. The distilled water used for watering the plants and for analytical purposes was obtained by distilling tap water through block tin pipes.

EXPERIMENTAL RESULTS

The data obtained from this experiment consist of those bearing upon the variations in the calcium and magnesium content of the bean plants at different growth stages when grown on different soil types, which show also the relationship of these variations to certain properties of the soils, and also of those related to the influence of the growth of the plants upon certain properties of the soils. Since a knowledge of the latter data is necessary to an understanding of the former, the latter are considered first in the following discussion.

Effect of the growth of beans upon certain characteristics of the soil, and the variations of different soil types in this regard

The marked influence of the growth of beans on the soil solution is shown in tables 1 and 2, where the calcium and magnesium contents, respectively, are given for the growing period of the plants.

Great differences were found to exist in the amounts of calcium present in the various soil solutions before plant growth started. The solutions of Roselawn loamy sand contained more than six times as much calcium as did the solution of Plainfield loamy sand, with the other soils solutions varying in between.

During the growth of the plant the calcium content of the soil solutions was reduced to a low level in which there was little variation. Usually the solutions of the soils which were highest in the beginning remained highest during the growth period.

Smaller amounts of magnesium than of calcium were present in the soil solutions. Although the range of difference in the soil types was small, it is evident that the soil solutions varied in the amounts of magnesium they

TABLE 1

Variations in the calcium content of soil solutions growing beans

Parts per million of water-free soil

SOIL TYPE	INITIAL Ca CONTENT	STAGES OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
Plainfield	10.60	12.50	2.68	2.16	5.05	4.00
Kewanee	44.70	24.60	30.40	......	16.10	....
Onaway	24.55	9.71	12.85	.....	.....	3.33
Roselawn	66.80	36.00	37.00	27.80	.....	8.95
Hillsdale	14.62	16.65	31.30	23.00	17.80	3.18
Brookston	19.10	.....	13.05	6.90	.....	3.33
Miami	30.06	26.90	26.62	.....	.....	6.89

TABLE 2

Variations in the magnesium content of soil solutions growing beans

(Parts per million of water-free soil)

SOIL TYPE	INITIAL CONTENT	STAGES OF GROWTH OF BEANS			
		3 weeks	6 weeks	Budding	Fruiting
Plainfield	4.44	3.089	Trace	Trace	0.7488
Kewanee	8.88	3.390	4.086	....	2.784
Onaway	7.45	2.056	1.938	....	Trace
Roselawn	9.84	3.900	3.354	....	1.114
Hillsdale	4.08	2.394	6.384	....	5.548
Brookston	3.48	0.926	1.566	....	1.600
Miami	10.20	5.810	5.352	....	....

contained; the soil solutions which were high in calcium generally were likewise high in magnesium.

No relationship appeared to exist between soil texture and the calcium or magnesium content of the soil solutions. The very sandy soils had either very high or very low calcium and magnesium contents in their solutions and the same was true of the fine textured soils.

The hydrogen-ion concentrations of the soils used are given in table 3, where it may be seen that a wide range of reaction existed, Miami soil being the most acid, with a pH value of 5.0 and Roselawn the most alkaline, with a pH

value of 7.8. No appreciable change occurred in the pH value of the soils during the growth period of the plants. The fluctuations appear to have been within the range of the influence of moisture and temperature changes and of biological activities.

Apparently there was no relationship between the pH values of the soils and the amounts of calcium and magnesium in the soil solutions.

Since it developed that the solutions of the soil types used varied in calcium and magnesium, in the order of Roselawn loamy sand, Kewanee sandy loam, Onaway sandy loam, Hillsdale sandy loam, and Plainfield loamy sand for the coarse-textured soils, and of Miami silt loam and Brookston loam for the fine-textured soils, and since the soil types fell into about this same order in respect to the extent that the calcium and magnesium contents of their solutions were maintained during the growth period, throughout the following work the soils will be considered as of decreasing strengths in this same sequence.

TABLE 3

Changes in pH values of soils growing beans

SOIL TYPE	INITIAL VALUE	STAGES OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
Plainfield	7.35	7.40	7.30	7.17	7.30	7.25
Kewanee	5.10	5.18	5.20	....	5.12	....
Onaway	7.40	7.27	7.36	....	7.37	7.42
Roselawn	7.85	7.87	7.87	7.72	8.00	7.80
Hillsdale	6.25	6.39	6.20	6.37	6.10	6.72
Brookston	7.05	6.94	7.08	6.66	6.75	....
Miami	5.00	5.10	4.60	....	....	5.64

Calcium content of green bean stems and leaves at different growth stages

The calcium content of green bean stems varied on the different soil types throughout the growth period, as is shown in table 4, where the soils are separated into light and heavy groups and then arranged according to decreasing amounts of calcium in their solutions at the beginning of the growth period. It is evident that the higher calcium contents occurred in green bean stems grown on soils with the greater amounts of calcium in their solutions. Some deviations from this appeared but the general tendency was very marked in this regard.

Considering that the calcium content of green bean stems was highest on Roselawn loamy sand and lowest on Plainfield loamy sand during the entire growth period, and that the stems grown on the very heavy soils contained about the same amount of calcium as stems grown on some of the very light soils, it appears that there was no relationship between soil texture and the amount of calcium in the green bean stems.

Similarly, the hydrogen-ion concentration appears to have borne no direct relationship to the amount of calcium in the green bean stems. Although the pH value of the Roselawn soil was highest and the calcium content of stems grown upon it was likewise highest, the pH value of the Plainfield soil was nearly as high, and yet the calcium content of stems grown upon this soil was lowest; Miami silt loam and Kewanee sandy loam had very low pH values and stems grown upon them had calcium contents about equal to the stems grown on soils with higher pH values, as Onaway sandy loam and Hillsdale sandy loam.

Larger percentages of calcium were present in the mature green bean stems than when the stems were young. The increase from early growth to maturity was not uniform and there generally appeared on each type an intermediate period during which little calcium was added to the stems, or during which calcium was actually removed. A large increase of calcium occurred in the stems grown on all soil types for which data were obtained except Brookston clay loam after the fruit had set.

TABLE 4

The effect of soil type on the calcium content of green bean stems at different stages of growth

SOIL TYPE	INITIAL Ca CONTENT OF SOIL SOLUTION	STAGES OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	66.8	0.253	0.4060	0.337	0.380	0.482
Kewanee	44.7	0.187	0 255	.....	0.351	.....
Onaway	24.5	0.202	0.230	.....	0.293	0.399
Hillsdale	14.6	0.243	0.225	0.267	0.263	0.422
Plainfield	10.6	0.162	0.220	0.216	0.234	0.336
Heavy soils						
Miami	30.0	0.207	0.200	.....	.....	0.434
Brookston	19.1	0.197	0.300	0.305	0.344	0.319

In table 5 are given the amounts of calcium present in the green material of bean leaves grown on different soil types. In this table the soils are arranged as in table 4.

The calcium content was much greater in green bean leaves grown on some soil types than in those grown on others. Here again, Roselawn soil generally gave leaves with the highest calcium content and Plainfield soil gave leaves with the lowest, and throughout the period of growth, a marked relationship was evident between the calcium content of the soil solution and that of the plant material. At each stage of growth, the calcium content of the green leaves grown on the different soil types, with few exceptions, varied directly as the decreasing amounts of calcium present in the initial soil solutions.

The calcium content of bean leaves was usually high on the soil types which gave stems with a high calcium content. Because of this, it is evident that there was no correlation between the texture or the pH values of the soils and the calcium content of bean leaves grown upon them.

A marked increase occurred in the calcium content of green bean leaves as the growth period advanced. The greatest increase in calcium occurred when the leaves were between three and six weeks old and between the time the fruit set and maturity. During the intermediate period, the increase in calcium was very slow or the calcium was even depressed. Much greater amounts of calcium were present in the green material of bean leaves than in that of bean stems throughout the growth period. A more rapid increase took place in the calcium content of the leaves than occurred in the stems, resulting in a wider difference between the calcium contents of the two plant parts at maturity.

TABLE 5

Effect of soil type upon calcium content of green bean leaves at different stages of growth

SOIL TYPE	INITIAL Ca CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	66.80	0.316	0.620	0.620	0.586	0.706
Kewanee	44.70	0.361	0.530	.....	0.566	.....
Onaway	24.55	0.360	0.538	.....	0.586	0.731
Hillsdale	14.62	0.266	0.503	0.483	0.421	0.722
Plainfield	10.60	0.249	0.434	0.403	0.411	0.564
Heavy soils						
Miami	30.06	0.448	0.658	0.400	.....	0.686
Brookston	19.10	0.301	0.476	0.450	0.556	0.601

Calcium content of the expressed juice of bean stems and leaves

Variations in the calcium content of the expressed juice of bean stems grown on different soil types are shown in table 6. Very similar differences can be seen in the calcium content of the expressed juice of bean stems as was evident in that of the green material. Generally the concentration of calcium in the juice was high in the stems which contained large amounts of calcium in their green tissue. Thus it follows that a high calcium content in the expressed juice of the bean stems was associated with a high calcium content in the soil solution. Deviations from this appeared on some of the soils at various times during the period of plant growth but still the correlation between the concentration of the calcium in the soil solution and the concentration of calcium in the expressed juice of the stems was plainly very high.

No closer relationship was evident between the texture nor the pH value of

the soil and the calcium content of the expressed juice of bean stems than was evident between these and the calcium content of the green material.

In table 7 is given the calcium content of the expressed juice of bean leaves at different stages of growth and on different soil types. Wide variations occurred in the calcium content of the juice of leaves grown on the different

TABLE 6

Effect of soil type upon the calcium content of the expressed juice of bean stems at different stages of growth

SOIL TYPE	INITIAL Ca CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	66.80	0.213	0.217	0232	0.240	0.288
Kewanee	44.70	0.200	0.205	.....	0.217	.....
Onaway	24.55	0.205	0.191	0.235	0.238	.....
Hillsdale	14.62	0.138	0.172	0.191	0.178	0.206
Plainfield	10.60	0.199	0.155	0.138	0.229	0.137
Heavy soils						
Miami	30.06	0.181	0..157	.....	.....	.....
Brookston	19.10	0.127	.....	0.170	.....	0.276

TABLE 7

Effect of soil type upon the calcium content of the expressed juice of bean leaves at different stages of growth

SOIL TYPE	INITIAL Ca CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	66.8	0.365	0.529	0.583	0.595	0.635
Kewanee	44.70	0.334	.....	.....	0.599	.....
Onaway	24.55	0.271	0.595	.....	0.598	0.690
Hillsdale	14.62	0.220	0.451	0.433	0.409	0.537
Plainfield	10.60	0.167	0.326	0.313	0.338	0.515
Heavy soils						
Miami	30.60	0.368	0.555	.....	.....	0.558
Brookston	19.10	0.189	0.348	0.413	.....	0.531

soil types throughout the period of growth. The relationship of the concentration of calcium in the soil solution to that of the expressed juice was less evident here than in the cases heretofore observed, although it still appears that the concentration of calcium in the plant juice was high where the concentration of calcium in the soil solution was high. The calcium contents of

the expressed juice of the leaves grown on Roselawn, Kewanee, and Onaway soils were very nearly equal regardless of marked differences in the amounts of calcium in the soil solutions.

Since there were marked similarities in the calcium contents of the juice of leaves grown on several of the soil types differing widely in texture and in hydrogen-ion concentration, it appears that there was no relationship between either the pH value or the texture of the soils and the calcium content of the juice of the bean leaves grown upon them.

In the juice of both stems and leaves there was an increase in the calcium content as the plants became more mature. The increase was more uniform in the juice of leaves than in that of the stems and it was much more rapid, so that in the mature stage there was a wide difference in the concentration of calcium in the juice of the two plant parts, there being more than twice as much in the juice of the leaves as in that of the stems.

TABLE 8

Effect of soil type upon the magnesium content of green bean stems at different stages of growth

SOIL TYPE	INITIAL Mg CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	9.84	0.044	0.029	0.052	0.083	0.0700
Kewanee	8.88	0.054	0.016	.....	0.026	......
Onaway	7.45	0.037	0.072	.....	0.079	0.0570
Plainfield	4.44	0.034	0.056	0.066	0.048	0.0792
Hillsdale	4.08	0.042	0.016	0.061	0.052	0.0570
Heavy soils						
Miami	10.20	0.058	0.064	.....	.....	0.0260
Brookston	3.48	0.062	0.061	0.100	0.086	0.0260

Magnesium content of green bean stems and leaves

The influence of soil types on the magnesium contents of green bean stems at different growth stages, is given in table 8, where the soil types are divided into light and heavy soils and then grouped according to decreasing amounts of magnesium in their solutions.

The magnesium content of green bean stems fluctuated greatly during the growing period on each of the soil types. Although noticeable differences occurred in the amounts of magnesium present in the green material at each stage of growth on the different soil types, there evidently was no relationship between the magnesium content and either the amount of magnesium present in the soil solution or the pH value of the soils as described earlier. However, it does appear that the magnesium content was higher in the stems of plants grown on the two heavy soil types than in those of the plants grown on the

light soil types. This last relationship did not hold when the plants had reached maturity.

On the light soil types there was more magnesium present in the green bean stems when the plants had reached maturity than when they were young, but during the intermediate growth stages a high point was always reached in which the magnesium content was greater than in the mature stage. On the heavy soil types the magnesium content of the stems was always lower at maturity then in the early stages of growth.

Much less magnesium than calcium was present in the green material of bean stems throughout the period of growth and the amount present fluctuated much more than the calcium.

A somewhat more uniform amount of magnesium was present in the green material of bean leaves than in that of the stems, as is evident in table 9.

TABLE 9

Effect of soil type upon the magnesium content of green bean leaves at different stages of growth

SOIL TYPE	INITIAL Mg CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	9.84	0.058	0.077	0.096	0.193	0.114
Kewanee	8.88	0.060	0.066	.....	0.075	.....
Onaway	7.45	0.068	0.077	.....	0.083	0.140
Plainfield	4.44	0.079	0.118	0.096	0.079	0.083
Hillsdale	4.08	0.055	0.105	0.083	0.075	0.105
Heavy soils						
Miami	10.02	0.082	0.099	.....	.....	0.079
Brookston	3.48	0.060	0.105	0.123	0.092	0.123

Greater amounts of magnesium were present at each stage of growth in the green material of leaves grown on some soil types than on others. Generally, in the early growth stages at least, there was more magnesium present in the leaves grown on soils with lower magnesium contents in their solutions. This was especially noticeable on the two heavy soils though this difference here might have been due to the low pH value of the Miami soil, a possibility which is supported by the fact that the plants grown on Kewanee soil also gave leaves with a low magnesium content. Further than this, no relationship appeared to exist between the characteristics of the soils and the magnesium content of the bean leaves.

Greater amounts of magnesium were present in the green bean leaves at maturity than when they were 3 weeks old in all plants except those grown on Miami soil. The increase of magnesium from early growth to maturity was generally not uniform. More magnesium was present in the leaves than

in the stems at all stages of growth and the rate of increase was somewhat faster. Much less magnesium than calcium was present in the leaves of beans throughout the growth period.

Magnesium content of the expressed juice of bean stems and leaves

In tables 10 and 11 are given the percentages of magnesium present in the expressed juice of bean stems and leaves at different stages of growth and on different soil types. Great fluctuation in the magnesium content was characteristic of the juice of both the stems and leaves of plants grown on different soil types and it appears that soil texture, the pH value of the soil, and the concentration of magnesium in the soil solution had no controlling influence on the concentration of magnesium in the plant juice.

TABLE 10

Effect of soil type upon the magnesium content of the expressed juice of bean stems at different stages of growth

SOIL TYPE	INITIAL Mg CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	9.84	0.041	0.031	0.067	.....	0.105
Kewanee	8.88	0.053	0.036	.....	0.048	.....
Onaway	7.45	0.043	0.035	.....	.....	0.115
Plainfield	4.44	0.037	0.055	0.050	0.099	0.155
Hillsdale	4.08	0.045	0.050	0.064	0.040	0.076
Heavy soils						
Miami	10.20	0.046	0.066	.....	.....	.....
Brookston	3.48	.....	.....	0.087	.....	0.060

Greater concentrations of magnesium were usually present in the juice of both stems and leaves as the plants advanced in age. On many of the soil types this increase of concentration of magnesium in the juice was quite uniform and it also was of considerable magnitude.

Magnesium was about equally concentrated in the juice of the stems and leaves throughout the growth period. This is in contrast with the concentration of calcium in the two plant parts, which was greater in the juice of the leaves than in that of the stems and which also increased much more rapidly in the juice of the leaves.

Total green plant material produced on the different soil types

The green plant material produced on each soil type at the various periods of growth is given in grams in table 12 and the corresponding proportion of stems and leaves in per cent is given in table 13.

TABLE 11

Effect of soil type upon the magnesium content of the expressed juice of bean leaves at different stages of growth

SOIL TYPE	INITIAL Mg CONTENT OF SOIL SOLUTION	STAGE OF GROWTH OF BEANS				
		3 weeks	6 weeks	Budding	Fruiting	Maturity
	p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roselawn	9.84	0.037	0.070	0.073	.....	0.098
Kewanee	8.88	0.024	.....	.....	0.031	.....
Onaway	7.45	0.057	0.088	.....	.....	0.109
Plainfield	4.44	0.052	0.061	0.091	0.076	0.042
Hillsdale	4.08	0.052	0.084	0.086	.....	0.088
Heavy soils						
Miami	10.20	0.057	0.122	.....	.....	0.075
Brookston	3.48	0.023	0.071	0.103	.....	0.105

TABLE 12

Green weight of beans at different stages of growth on different soil types

(Weight of 7 plants in grams)

SOIL TYPE	STAGE OF GROWTH OF BEANS				
	3 weeks	6 weeks	Budding	Fruiting	Maturity
Plainfield	9.94	32.5	33.25	42.0	40.0
Kewanee	10.20	16.0	.....	38.0	....
Onaway	10.50	19.8	.....	38.0	38.0
Roselawn	9.80	16.1	41.00	43.0	56.0
Hillsdale	12.88	26.0	45.50	46.5	55.0
Brookston	11.20	19.5	29.00	31.0	22.5
Miami	10.29	16.5	.....	....	23.5

TABLE 13

Proportions of leaves and stems of beans at different stages of growth on different soil types

SOIL TYPE	STATE OF GROWTH OF BEANS									
	3 weeks		6 weeks		Budding		Fruiting		Maturity	
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield	47.3	52.7	38.4	61.6	39.8	60.2	30.8	69.2	32.1	67.9
Kewanee	....	....	34.4	65.6	....	....	34.2	65.7	....	....
Onaway	45.0	55.0	35.8	64.2	....	....	36.0	64.0	31.6	68.4
Roselawn	43.0	57.0	33.0	67.0	35.3	64.7	36.0	64.0	28.5	71.5
Hillsdale	43.8	56.2	55.4	44.6	38.5	61.5	37.9	62.1	31.8	68.2
Brookston	47.7	52.3	35.8	64.2	34.5	65.5	32.2	67.9	35.5	64.5
Miami	47.4	52.6	39.3	60.7	....	....	....	....	36.1	63.9

Considerably better growth was made on some of the soil types than on others. The growth was usually light on the very heavy soils and also on the very acid soils and apparently was independent of the amount of calcium and magnesium present in the soil solutions. There is no evidence that there was any relationship between the rate of growth and the calcium or magnesium content of the plant material.

Proportion of stems and leaves of the bean plant

Some variance occurred in the proportions of stems and leaves produced on the different soil types at each stage of growth but it was small and does not appear to depend on the rate of growth nor upon the characteristics of the soil which are considered here. The proportion of stems is less than that of leaves during the entire period of growth. At the beginning of growth, the difference in the proportions was small, averaging 45.7 per cent stems and 54.3 per cent leaves. The proportion of leaves increased as the growth period advanced, with the result that at maturity the proportions were 32.6 per cent of stems and 67.4 per cent of leaves.

A comparison of calcium and magnesium contents of cropped and uncropped soils

Depressions in the amount of calcium and magnesium in the soil solutions, as was observed in this work, may not have been entirely due to the growth of the crop. To check this point, two series of pots containing Plainfield and Hillsdale soils were left fallow and sampled at the same time that two other series, containing the same soils, but growing beans, were analyzed. The results obtained are given in table 14. Here it will be seen that there were fluctuations in the amounts of calcium and magnesium present in the soil solutions of both the cropped and uncropped soils, but the general tendency was for the concentration of the elements in the solutions to decrease on the cropped soils and to hold about constant on the uncropped soils.

Power of soils to rebuild their solutions when air-dry

The noticeable reduction in the concentration of the soil solution due to the growth of plants (9, 23) indicates that during a period of rest soils must be able to rebuild their solutions in order for plant growth to be possible year after year. In the work presented here, two soils previously depleted in the greenhouse, were allowed to rest for 82 days and their solutions obtained and analyzed. The data obtained are given in table 15 where it will be seen that in the case of calcium, both Plainfield and Hillsdale soils were able to rebuild their solutions above what they were before crop growth started. These soils were held as air-dry under greenhouse conditions.

The magnesium content of the soil solutions was likewise rebuilt during the period of rest and attained a level higher than that possessed before the beginning of plant growth. Since the calcium content is reduced on Hillsdale to a lower level that on Plainfield, by the growing crop and yet after the rest

period has rebuilt its solution to contain more than three times as much calcium, it would appear that Hillsdale has more ability to rebuild its solution than has Plainfield.

Although this result is in agreement with that of Duley (7), this increase of solutes in the soil solution might perhaps be attributed to the wetting and drying of the soil. It is a question as to whether a soil maintained in the dry condition can actually increase the amount of solutes available for solution. Unfortunately this question did not present itself until too late to obtain data bearing upon it.

TABLE 14

Calcium and magnesium content of cropped and uncropped soils

Parts per million of moisture-free soil

SOIL TYPE	CALCIUM				MAGNESIUM			
	Initial content	Stage of growth			Initial content	Stage of growth		
		10 days	24 days	35 days		10 days	24 days	35 days
Plainfield cropped	17.87	8.51	1.44	1.74	6.67	2.12	9.16	1.46
Plainfield uncropped	17.81	11.91	16.30	11.15	6.67	4.99	1.57	8.73
Hillsdale cropped	38.15	32.80	12.86	4.04	12.02	11.50	1.22	0.80
Hillsdale uncropped	38.15	45.80	39.90	50.30	12.02	11.34	9.57	9.62

TABLE 15

Power of soils to rebuild their solution when dry

SOIL TYPE	CALCIUM			MAGNESIUM		
	At start of growth period	At end of growth period	After resting 82 days	At start of growth period	At end of growth period	After resting 82 days
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Plainfield	10.60	4.00	11.58	4.44	0.7488	6.783
Hillsdale	14.62	3.18	35.48	4.08	1.7100	10.996

SUMMARY AND CONCLUSIONS

In this work the effect of crop growth upon the calcium and magnesium content of the soil solutions and upon the pH value of the soils was observed, as were also the relationships between these characteristics of the soils and the calcium and magnesium content of the plants. Variations occurring in the calcium and magnesium contents of green bean stems and leaves and in their expressed juice when obtained from plants of different ages grown on a number of soil types were studied. The relationship of soil texture to the foregoing characteristics of the plants was noted.

Widely different amounts of calcium and magnesium were found present in the various soil solutions before plant growth began but they were greatly reduced by the growing plants and were almost equal at the end of the growth

period. The soil solutions with the highest concentrations of calcium and magnesium at the beginning of the growth period generally maintained a higher concentration during the growing period.

Insignificant variations appeared in the pH values of the different soils as the growth period advanced but they did not appear to result from the growth of the plants.

No relationship appeared to exist between the calcium and magnesium content of the soil solutions and either the pH value or the texture of the soils.

Marked variations were found in the amounts of calcium and magnesium present in the green tissue and in the juice of both stems and leaves of bean plants grown on the different soil types.

An increase generally occurred in the calcium and magnesium contents of the tissue and of the juice of stems and leaves as the growth period advanced. Some minor exceptions occurred.

The calcium content was always greater than the magnesium content. The calcium and magnesium contents of the leaf tissue were always greater than those of the stem tissue. The calcium content of the juice of leaves was always greater than that of the juice of stems but the magnesium content of the juice was sometimes greater in the stems and sometimes greater in the leaves.

Greater increases in the calcium and magnesium contents of the tissue and juice of stems and leaves generally occurred in early growth and near maturity than during the intermediate stages of growth.

A very decided correlation appeared to exist between the calcium content of the tissue and juice of both stems and leaves and that of the soil solution. A high calcium content in the soil solution was associated with a high calcium content in the plants when the soils were of similar texture.

No correlation appeared to exist between the calcium content of the plants as studied and either the texture or the pH value of the soil.

The variations in the magnesium content of the plants were so inconsistent that no correlations could be drawn between them and the magnesium content of the soil solutions, the textures of the soils, or their pH values.

There appeared to be no relationship between the rate of growth and either the calcium or the magnesium content of the plants.

There were slight variations in the proportions of stems and leaves on different soil types and they did not appear to depend on any of the properties of the soils studied. The proportion of leaves was always greater than that of stems and the ratio became wider as the period of growth advanced.

The calcium and magnesium in the soil solutions were greatly reduced in the soils growing plants, as compared with the solutions of the uncropped duplicate soils.

Plainfield and Hillsdale soils were able greatly to rebuild the calcium and magnesium contents of their solutions when kept air-dry in the greenhouse for 82 days. The Hillsdale soil surpassed the Plainfield in this respect.

REFERENCES

(1) Austin, R. H. 1928 The effect of soil type and fertilizer treatment on the composition of the soybean plant. Thesis Mich. State Col.

(2) Baver, L. D. 1925 The use of the quinhydrone electrode for measuring the hydrogen-ion concentration of soils. *Soil Sci.* 21: 167–179.

(3) Billman, E., and Tovborg-Jenson, S. 1927 On the determination of the reaction of soils by means of the quinhydrone electrode. *Trans. Second Comn. Internat. Soc. Soil Sci.* B: 236–274.

(4) Burd, J. S. 1918 Water extraction of soils as criteria of their crop producing powers. *Jour. Agr. Res.* 12: 297–309.

(5) Burd, J. S. 1919 Rate of absorption of soil constituents at successive stages of plant growth. *Jour. Agr. Res.* 18: 51–72.

(6) Burd, J. S., and Martin, J. C. 1927 Water displacement of soils and the soil solution. *Jour. Agr. Sci.* 13: 265–295.

(7) Duley, F. L. 1923 Easily soluble calcium of the soil in relation to acidity and returns from liming. *Soil Sci.* 17: 213–228.

(8) Fleetwood, J. R. 1925 Easily soluble calcium of soils as an indicator of their response to liming. *Soil Sci.* 19: 441–457.

(9) Fonder, J. F. 1928 A critical study of the effect of soil type upon the calcium and magnesium content and other physiological characteristics of the alfalfa plant. Part of a thesis presented to the Graduate Committee of Michigan State College.

(10) Gilbert, N. E., and Hardin, L. J. 1927 The current mineral nutrient content of the plant solution as a possible means of chemical control of optimun fertilization. *Jour. Agr. Res.* 35: 185–192.

(11) Handy, J. O. 1900 The volumetric determination of magnesium. *Jour. Amer. Chem. Soc.* 22: 31–39

(12) Hibbard, P. L. 1923 Comparison of the soil solution by displacement method and the water extract of alkali soils. *Soil Sci.* 16: 465–471.

(13) Hoagland, D. R. 1918 The freezing point method as an index of variations in the soil solution due to season and crop growth. *Jour. Agr. Res.* 12: 369–395.

(14) Hoagland, D. R., and Martin, J. C., and Stewart, G. R. 1920 The relation of the soil solution to the soil extract. *Jour. Agr. Res.* 20: 381–395.

(15) Kelley, W. P. 1926 Base exchange in soils. *Jour. Amer. Soc. Agr.* 18: 450.

(16) McCool, M. M. 1926 Relation of soil to plant cell sap. *Mich. Agr. Exp. Sta. Quart. Bul.*, 9: 60–64.

(17) McCool, M. M. 1927 The effect of soil fertilization on the moisture content, density, heat of wetting, and phosphorus content of the cell sap of plants. *Proc. 1st Internat. Soil Sci. Congr.*

(18) McCool, M. M., and Millar, C. E. 1917 The water content of the soil and the composition and concentration of the soil solution as indicated by the freezing point lowerings of the roots and tops of plants. *Soil Sci.* 3: 113–138.

(19) McCool, M. M., and Weldon, M. D. 1928 The effect of soil type and fertilization on the composition of the expressed sap of plants. *Jour. Amer. Soc. Agron.* 20: 802–807.

(20) Parker, F. W. 1921 Methods of studying the concentration and composition of the soil solution. *Soil Sci.* 12: 209–232.

(21) Parker, F. W., and Tidmore, J. W. 1926 The influence of lime and phosphatic fertilizers on the phosphorus content of the soil solution and of soil extracts. *Soil Sci.* 21: 425–441.

(22) Russell, E. J. 1927 Soil Conditions and Plant Growth. London.

(23) Stewart, G. R. 1918 The effect of season and crop growth in modifying the soil extract. *Jour. Agr. Res.* 12, 6: 311–368.

(24) Swansen, C. O., Gainey, P. L., and Latshaw, W. L. 1924 Relation of calcium in soil to absolute reaction. *Soil Sci.* 17: 181–191.

SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: III. INFLUENCE OF THE STAGE OF PLANT GROWTH UPON SOME ACTIVITIES OF THE ORGANISMS

ROBERT L. STARKEY

New Jersey Agricultural Experiment Station

Received for publication January 30, 1929

Previous reports (6, 7) have shown that the members of the soil population are greatly affected by the development of higher plants. Although all plants may affect certain of the organisms, different plants behave differently and the effects of any one plant vary with the stage of growth.

It seems likely that certain of the activities of the organisms in the soil should be affected in a manner similar to numbers of the cells since the detection of modifications in the soil population suggests that there are modifications in the transformation of such soil constituents as are associated with the nutrition of this population.

Stoklasa's results (8) showed quite clearly that, at a certain arbitrary period of measurement, the evolution of carbon dioxide from soils supporting different plants was different. It appears logical to question whether similar relationships would exist at all periods of plant growth, particularly where both annuals and biennials are involved.

It is quite natural to expect that greater amounts of carbon dioxide would be evolved from plants as they become larger (5). However, it may not be accurate to assume that the increase in carbon dioxide in the soil, a change associated with plant growth, is entirely due to excretion of carbon dioxide from the roots. Soil microörganisms may be more concerned with this change than was assumed by Barakov (1) and Turpin (9) and at least some of the increase in bicarbonates about plant roots (4) may arise from microbial activity in the rhizosphere.

Lyon and Bizzell (3) demonstrated that nitrification proceeded at different rates in soils during different periods of growth of higher plants. During the early stages of plant growth when the crops were making their greatest drafts upon the nitrogen in the soil, nitrification was most rapid and even proceeded with greater rapidity than in fallow soil at these periods. The most marked acceleration appeared under legumes. This suggests that there may be some differences in the influences of the plants upon soil activities at different periods of plant development.

The following studies are concerned with the influences which higher plants exert upon the rate of decomposition of soil organic matter as measured by the

evolution of carbon dioxide from the soil and upon the reactions concerned with the formation of nitrates in the soil.

EXPERIMENTAL PROCEDURE

These experiments were conducted simultaneously with the studies on abundance of microörganisms under higher plants as outlines in the preceding report of this series (7). Observations were made upon soils supporting growth of plants both in the greenhouse and in the field. Soils which supported no higher plants were periodically compared with soils which were gathered from plant roots.

In determining the evolution of carbon dioxide, duplicate samples of the moist soils, which were equivalent to 100 gm. of oven-dry soil, were placed in containers, brought to the same moisture content and introduced into an apparatus (11) for determining the amount of carbon dioxide which was generated in a period of 15 days at a temperature of 27°C. Only averages of the results upon the duplicate samples of soil are reported.

Nitrification studies were made only upon the field soils. They were made in two of the ways suggested by Waksman (10). In one case, samples of the moist soils, equivalent to 100 gm. of oven-dry soil, were placed in tumblers, brought to the same moisture content, covered, and incubated at 27 °C. Occasionally water was added to the soils to bring the moisture content to its original condition. At intervals varying from 5 to 30 days, determinations were made for ammoniacal and nitrate nitrogen. In the second case, nitrification of added ammonium sulfate was determined. To samples of the moist soils equivalent to 100 gm. of oven-dry soil, 210 mgm. of calcium carbonate were added and uniformly distributed throughout the soil. Some ammonium sulfate was dissolved in distilled water and an amount of the solution containing 30 mgm. of nitrogen was sprinkled over the soil and carefully mixed into it. These soils were incubated at 27°C. and periodically studied for the presence of ammonical and nitrate nitrogen.

The determinations for ammonia were made according to a method of Bengtsson (2). Extracts of the soil which were made with normal potassium chloride were distilled in the presence of magnesium oxide into standard acid which was titrated back with standard alkali. Nitrate determinations were made by the phenoldisulfonic acid method.

EXPERIMENTAL RESULTS

Evolution of carbon dioxide

Among the activities associated with microbial development, the production of carbon dioxide is one of the few which is common to practically all organisms and lends itself readily to measurement in soils. Under aerobic conditions, in a mixed population, it is the principal carbonaceous end product of decomposition. Results of determinations for the carbon dioxide which is

liberated from soils may be interpreted as indicating fairly accurately the speeds of decomposition of the organic materials in the soils. The method is much more accurate for estimating some of the influences of plant development upon the soil organisms than counts of the abundance of organisms by the plate method. Consequently, small differences in the amounts of carbon dioxide formed from the various soils may be considered as being significant where similar differences in the determined abundance of microörganisms would be within the error of the determination.

TABLE 1

*Carbon dioxide evolved from fallow soils and from soils obtained from the roots of various plants**

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Field soils						
Fallow	27.7	18.4	16.2	15.7	15.0	18.6
Oats	22.9	26.5	22.4	17.0	17.2	21.2
Corn	28.4	27.3	23.9	22.4	18.9	24.2
Beans	28.7	24.1	19.0	20.2	19.3	22.3
Potatoes	23.1	23.0	18.8	18.7	15.7	19.9
Table beets	21.1	23.0	20.5	20.1	28.0	22.5
Mangel beets	26.2	27.1	21.1	23.0	24.7	24.4
Rape	26.0	28.4	24.2	24.5	28.0	26.2
Sweet clover†	22.8	20.2	17.1	18.5	29.5	21.6

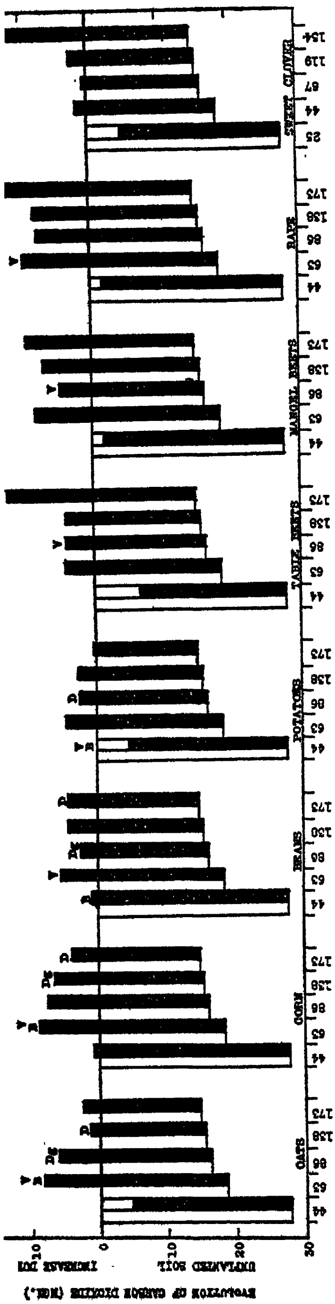
Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	10.3	8.7	10.8	9.6	8.2	10.6	9.7
Oats	12.8	10.1	12.6	16.9	15.2	14.8	13.7
Beans	10.5	12.1	11.0	10.9	10.2	11.3	11.0
Beets	10.7	10.4	10.6	13.9	13.7	11.7	11.8
Rape	11.2	11.0	12.2	15.6	11.9	13.4	12.6
Sweet clover	10.4	13.1	11.7	11.8	11.6	11.1	11.6

* Mgm. of carbon evolved as carbon dioxide from the equivalent of 100 gm. of dry soil in 15 days.

† For sweet clover the sampling periods are 25, 44, 67, 119 and 154 days.

Averages of the results of these studies are presented in table 1 and figure 1. Only the results of the field studies are presented graphically. From the arrangement of the data on the graph, the degree of the extension of the shaded columns above the zero line indicates the amounts of increases in evolution of carbon dioxide due to plant growth. The letters at the tops of the columns in the figure refer to the stages of plant growth: *B*—blooming, *V*—height of vegetative development, *Dg*—initial vegetative degeneration, *D*—death.



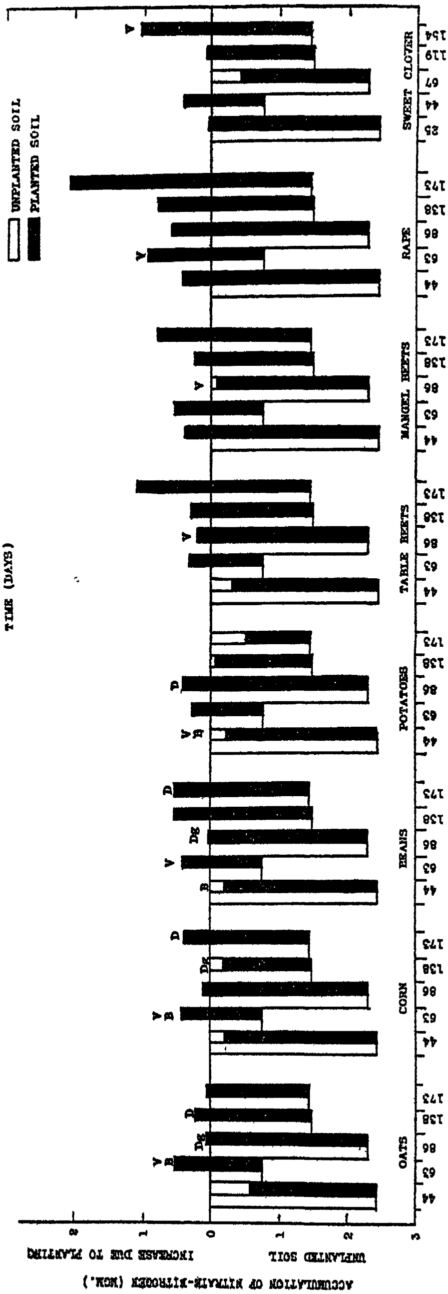


FIG. 1 ABOVE. INFLUENCE OF PLANT DEVELOPMENT UPON THE EVOLUTION OF CARBON DIOXIDE FROM THE SOIL

FIG. 2 BELOW. INFLUENCE OF PLANT DEVELOPMENT UPON THE CAPACITY OF SOILS TO FORM NITRATE FROM THE SOIL NITROGEN

(Letters at the tops of the columns refer to stages of plant growth: *B*—blooming, *V*—height of vegetative development, *Dg*—initial degeneration, *D*—death)

There are many features of these results which show similarities to the changes in abundance of the bacterial population and organisms of the *B. radiobacter* group reported previously (7).

All of the plants caused increases in the evolution of carbon dioxide but the course of the formation of the gas appears to be distinct for each of the plants and related to differences in characteristics of growth of the plants, particularly of the plants in the field. All plants influence the transformation to a slight degree in the early stages of growth. In fact, in many cases at the first period of sampling, the planted soils gave off even less carbon dioxide than the unplanted soils. It was quite striking in many cases, what an abundance of plant roots might permeate the soils in early stages of plant growth without exerting any noticeable effects upon either the abundance of organisms or the evolution of carbon dioxide. The plants may be divided into two distinct groups on the basis of their influences upon carbon dioxide production subsequent to the initial period of sampling. The first group includes plants which mature in a single year. The second group includes plants which require more than one year to mature. The first group is represented by oats, corn, beans, and potatoes. These plants in all cases produced the most marked influence upon evolution of carbon dioxide at the second period of sampling. At this time these plants were well advanced in growth and were either at maximum vegetative development or had passed this stage. Subsequent to this period, during phases of degeneration, the influences upon formation of carbon dioxide were less pronounced. The plants exerted even less influence upon the process subsequent to their death.

The second group of plants is represented by table beets, mangel beets, rape, and sweet clover. Even though the formation of carbon dioxide was greatly increased under these plants subsequent to the first period of sampling, this favorable influence did not decrease as time advanced. In fact, at the time of the last sampling the effects were greater than at any previous period. This was particularly striking in the case of the sweet clover. The fact that these biennials were as vigorous in vegetative development at the final period of sampling as at any previous period is undoubtedly largely responsible for the continued high level of formation of carbon dioxide under these plants.

It is quite apparent that the formation of carbon dioxide in the unplanted soil in the field decreased progressively throughout the period of study. This suggests that the decomposable organic matter in unplanted soil becomes depleted to a marked degree within a single season.

Whatever factors are responsible for increasing the production of carbon dioxide in soils under plants, continue to be active as long as the plants are developing. The factors are apparently regulated by physiological characteristics associated with growth and senescence of the plants.

It seems particularly suggestive that microörganisms may evolve considerably greater amounts of carbon dioxide from soils supporting root growth than

from soils containing no roots. This is particularly significant in view of the fact that this increase is associated with periods of active development of the plants, particularly the periods of maximum vegetative development, blooming or early vegetative degeneration. Since this is the case it would seem to be logical to conclude that at least part of the increase in carbon dioxide in the soil air under plants and increase in bicarbonates about plant roots should be ascribed to microbial action and not entirely to respiration of the plant roots as assumed by Barakov, Turpin, and Metzger. What portion of the total increase in carbon dioxide under plants is due to the microörganisms is uncertain, this may be a relatively slight or large proportional amount. At least, formation of carbon dioxide by the microörganisms follows much the same course of changes as the total increases and decreases of the gas under plants.

As regards the importance of microörganisms about plant roots as factors affecting the feeding power of plants, it appears likely that with annuals they exert very lttle greater effects upon the solubility of nutrients in soils supporting plants than in soils supporting no plants. This appears probable in view of the fact that the acceleration of biological activities, being dependent upon contributions from the plants, becomes progressively greater as the plants mature, and reaches its highest point only at periods subsequent to maximum vegetative development. The requirements of the plants for soil nutrients follow an inverse course, being greater in the early stages of development and decreasing when microbial activities appear to be greatest. Under plants developing over longer periods of time, as in the case of biennials, the microbial activities are at a high level long before flowering and seed development and may exert much greater effects upon the solubility of soil nutrients about roots than in fallow soils during vegetative growth. Even in these instances, however, maximum vegetative development and consequently absorption of nutrients, occur before the most pronounced effects of the plants are exerted upon the microörganisms.

Nitrification of the soil nitrogen

The data presented in table 2 and figure 2 indicate the amounts of nitrate-nitrogen formed during the periods of incubation indicated in the table. The data represent the differences between the amounts of nitrate-nitrogen found in the soils at the termination of the incubation period and the amounts present at the time that the soils were sampled. The results from the soils sampled at the second period are not directly comparable with the others since they represent nitrate formation during a shorter period of time.

Although nitrate formation from these soils was not influenced to as great a proportional degree by plant development as were the numbers of certain groups of bacteria, it was significantly modified and in somewhat the same manner as the evolution of carbon dioxide. At the first period of sampling,

some of the biennials were the only plants which brought about greater nitrification in the soil than occurred in the absence of plant growth. The influence of the annuals was erratic at subsequent periods of study, but in most cases while the plants were alive, the accumulation of nitrates was greater in the soils which supported root growth. The formation of nitrates appeared to be affected by the beans to a greater extent and more consistently than by any of the other annuals. As with their influence upon the bacterial population, the potatoes showed less extended favorable effects upon nitrification than any of the other plants. This may be correlated with the short period of growth of the potatoes.

Nitrification was much more active in soils under biennials than under annuals and the duration of this effect extended throughout the season. At the last period, nitrification was greater under the biennials than at any previous period with any of the plants. Rape exerted the most pronounced effects throughout the study. Although the sweet clover showed little effect until the last sampling, this may be because this plant grows more slowly than any of the other plants and had only reached the stage of vigorous and abundant vegetative growth at the end of the season.

TABLE 2

*Accumulation of nitrate-nitrogen in fallow soil and in soils obtained from the roots of various plants**

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	2.44	0.75	2.29	1.49	1.46	1.69
Oats	1.87	1.30	2.37	1.73	1.51	1.76
Corn	2.24	1.19	2.41	1.33	1.85	1.80
Beans	2.26	1.16	2.31	2.05	2.02	1.96
Potatoes	2.24	1.03	2.72	1.47	0.98	1.69
Table beets	2.15	1.11	2.52	1.83	2,57	2.04
Mangel beets	2.84	1.32	2.23	1.76	2.29	2.09
Rape	2.87	1.67	2.90	2.30	3.56	2.66
Sweet clover†	2.48	1.15	1.88	1.58	2.48	1.91
Period of incubation (days)	30	18	31	28	30	....

* Mgm. of nitrate-nitrogen per 100 gm. of dry soil.

† For sweet clover the incubation periods are 25,44, 67, 119, and 154 days.

It seems justifiable to conclude that accumulation of nitrates is more rapid in soils obtained from plant roots than from soils supporting no plant growth. At least such is the case at certain stages in the growth of all the plants which were studied. It is not clearly apparent from these observations what differences are exerted by the plants at different stages of growth except that the greatest accumulation occurs during periods of growth of the plants and consequently persists for longer times under biennials than annuals. No marked differences were observed between the action of legumes and non-legumes

which could not be explained as being correlated with differences in duration of vegetative growth. It is quite possible that the development of higher plants increases the abundance of nitrifying organisms in soils although the method which was used for studying nitrification is not devised to detect such effects. An increase in the rapidity of accumulation of nitrates by this method of study does indicate, however, that certain portions of the soil organic matter

TABLE 3

Disappearance of ammoniacal-nitrogen and formation of nitrate-nitrogen from ammonium sulfate added to fallow soil and soils obtained from the roots of various plants

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
*Ammoniacal-nitrogen consumed**						
Fallow	29.9	27.0	10.8	5.6	13.6	17.4
Oats	30.1	29.7	10.4	7.3	14.8	18.5
Corn	30.1	27.5	10.4	8.6	16.2	18.6
Beans	29.6	26.9	12.1	10.0	17.2	19.2
Potatoes	29.3	27.3	10.0	6.6	13.6	17.4
Table beets	28.2	24.9	12.9	9.1	15.0	18.0
Mangel beets	29.3	26.3	12.0	9.4	14.9	18.4
Rape	30.1	27.7	13.1	10.4	15.1	19.3
Sweet clover†	29.7	26.6	10.2	8.9	14.4	18.0
*Nitrate-nitrogen formed**						
Fallow	30.38	23.86	7.59	4.90	12.47	15.84
Oats	28.60	25.05	8.20	5.08	13.60	16.11
Corn	31.82	22.67	4.55	6.11	13.45	15.72
Beans	29.46	23.15	10.39	6.88	14.03	16.78
Potatoes	27.63	23.09	8.29	5.64	12.01	15.33
Table beets	27.75	20.10	8.45	6.71	13.13	15.23
Mangel beets	27.26	21.18	9.75	7.20	13.07	15.69
Rape	29.43	24.05	10.67	7.17	17.07	17.68
Sweet clover†	27.33	21.83	8.44	5.57	13.39	15.31
Period of incubation (days)	30	18	5	5	5	....

* Mgm. of nitrogen per 100 gm. of dry soil.

† For sweet clover the incubation periods are 25, 44, 67, 119 and 154 days.

are undergoing more rapid decomposition and that these organic materials are of fairly narrow carbon-nitrogen ratios. In view of these assumptions it appears likely that the plants have added this organic matter to the soil. Since the effects appear previous to the death of the plants it seems likely that considerable amounts of such organic materials may be added to the soil during certain phases of root development. These conclusions are further emphasized by the results of the observations upon the abundance of the microörganisms

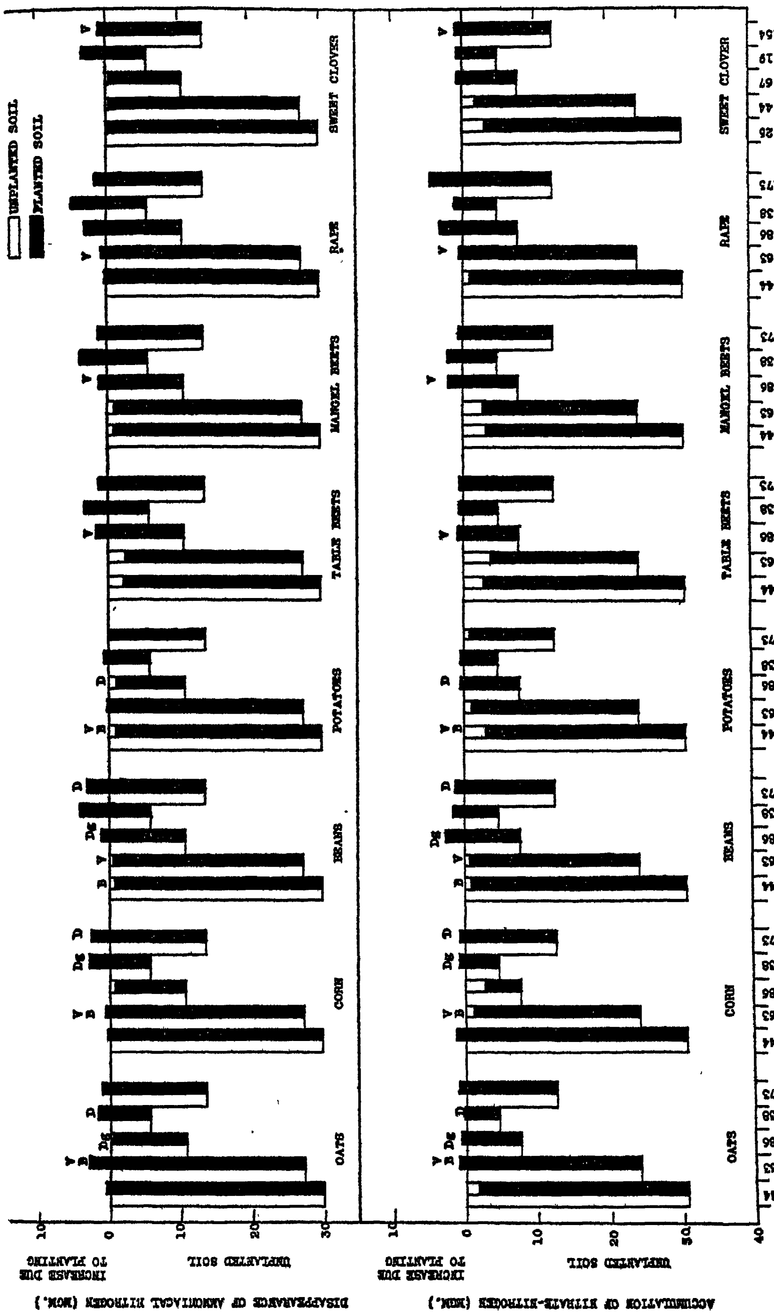


FIG. 3. INFLUENCE OF PLANT DEVELOPMENT UPON THE CAPACITY OF SOILS TO TRANSFORM AMMONIACAL-NITROGEN

(Letters at the tops of the columns refer to stages of plant growth: *B*—blooming, *V*—height of vegetative development, *Dg*—initial degeneration, *D*—death)

in the soils (7), and upon the formations of carbon dioxide from the soils. These studies seem to suggest that during the time when plants are developing in the field, nitrification proceeds at a more rapid rate about the roots at certain stages in their development than in fallow soils, in spite of the fact that greater amounts of nitrate-nitrogen are detected in fallow soils.

If the second period of sampling is left out of consideration (this appears justified since the incubation period was much shorter than for the other soils) it seems clearly apparent from the figure that accumulation of nitrate-nitrogen in the unplanted soil becomes slower with the passage of time. At the first sampling, 2.44 mgm. of nitrate-nitrogen were formed in 30 days. At the last sampling, 129 days later, only 1.46 mgm. were produced.

Nitrification of ammonium sulfate

Nitrification of ammoniacal-nitrogen added to soils may be determined by measuring both the amounts of nitrate-nitrogen formed and the ammoniacal-nitrogen consumed after certain periods of incubation. Both methods have been used in the present study. The data are presented in table 3 and figure 3. During the first observations the soils were incubated for 30 days before the extent of nitrification was determined. This proved to be too long a period of time since practically all of the ammoniacal-nitrogen was oxidized to nitrate-nitrogen and any differences which might have existed between the planted and unplanted soils were obscured. Even an incubation period of 18 days, which was used with the soils at the second sampling, proved too long. Nitrification in the soils of the last three samplings was determined after incubation for 5 days. Results of these last observations show some slight influences of development of plants upon nitrification of ammonia. Greater nitrification is apparent if the process is measured by disappearance of ammonia than by formation of nitrate. In fact, except with the beans, mangel beets, and rape, it would appear that the plants exerted no detectable influence on nitrification of ammonium sulfate as measured by formation of nitrate. On the other hand, if disappearance of ammonia is considered, it appears that in the last three periods of study all of the plants except potatoes increased nitrification. The explanation for the differences between nitrate formation and ammonia disappearance does not appear to be clear. It might be assumed that the development of higher plants introduced into the soils appreciable amounts of decomposable organic material containing very little nitrogen. In the decomposition of such substances microörganisms would assimilate some of the available ammoniacal nitrogen. However, such an explanation does not seem logical in view of the fact that nitrate-nitrogen accumulated in considerable amounts during the incubation of these soils where no substances were added. It is quite possible that some of the ammonia or nitrate became physically attached in the soil and was not detected by the

methods used for its estimation. Whatever factors were active, exerted a more pronounced effect in the planted soils.

Provided that nitrification of ammonium sulfate was more rapid in soils obtained from plant roots than from fallow soils it seems likely that this might be ascribed to the presence of a more active nitrifying population. The moisture content of all of the soils was kept the same, the temperatures of incubation were identical and the reactions were practically the same in all cases. Assuming that the activity of the organisms at the time of sampling was the controlling factor, it is reasonable to expect that differences between the soils would be eliminated after prolonged incubation subsequent to the addition of ammonium sulfate. Differences would be expected only in the first few days after the addition of ammonia. Such has proved to be the case. Although no conclusions can be drawn concerning the periods at which nitrification is most rapid or concerning the factors involved, it does appear that development of practically all of the plants has resulted in the appearance of a more active nitrifying flora. As in the other biological studies, the potatoes produced the slightest effects and the rape the most marked influences.

SUMMARY

Results are reported of the influences of the development of higher plants upon certain activities of microörganisms in soils. Measurements were made of the amounts of carbon dioxide formed by the microörganisms, of the amounts of nitrate-nitrogen produced from the soil organic matter, and of the nitrification of ammonium sulfate. The measurements were made periodically during the growth of plants cultivated both in the field and greenhouse. The following effects were apparent:

1. The evolution of carbon dioxide was greater from soils which supported plant growth and the course of the formation of the gas during the season was distinct for each of the plants and related to growth characteristics of the plants.
2. The course of the influences of the plants on the formation of carbon dioxide was much the same as the course of changes in the bacterial population in the soil. The plants exerted slight effects in the early stages of growth, maximum effects at advanced vegetative development and fruiting, and less pronounced effects subsequent to degeneration and death. Because of their more prolonged development, biennials raised the level of carbon dioxide production over longer periods of time than did the annuals.
3. The evolution of carbon dioxide in unplanted soil decreased as the season advanced.
4. Nitrification of the soil nitrogen was affected in somewhat the same manner as was evolution of carbon dioxide. Nitrates accumulated more rapidly in soils which supported growth of plants, and the enhanced effects of plants were apparent during advanced stages of growth.
5. Nitrification of ammonium sulfate in the soils did not appear to be affected by plant development to so great an extent as nitrification of the soil nitrogen. During the early stages of the transformation, ammonia disappeared more rapidly in the soils which supported plant growth but the differences became obscured during extended periods of incubation.

The influences of plants upon this process appear to be more striking if the transformation is studied by determining the disappearance of ammonia than by determining the formation of nitrate.

6. The acceleration in evolution of carbon dioxide and nitrification of the soil nitrogen is believed to be the result of the addition of organic substances to the soil by the growing plants. It seems likely that these organic materials have relatively narrow carbon-nitrogen ratios. Transformation of these plant materials may be responsible for increasing the activity of the nitrifying flora.

7. The course of the influences of the plants upon the evolution of carbon dioxide by the soil organisms suggests that the microörganisms are important agents in increasing the carbon dioxide in the soil and the bicarbonates about roots during plant development.

8. The plants increase the activities of soil organisms to a pronounced degree after the periods of maximum absorption of nutrients by the plants. Consequently the availability of the nutrients in fallow soils may be much the same as their availability in planted soils during the periods when these substances are required in greatest abundance by the growing plants.

REFERENCES

(1) Barakov, P. 1910 The carbon dioxide content of soils during different stages of growth of plants. *Zhur. Opuytn. Agron.* (*Russian Jour. Exp. Landw.*) 11: 321–343. (See *Exp. Sta. Rec.* 23: 523).

(2) Bengtsson, N. 1924 The determination of ammonia in soil. *Soil Sci.* 18: 255–278.

(3) Lyon, T. L., and Bizzell, J. A. 1913 Some relations of certain higher plants to the formation of nitrates in soils. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 1: 1–111.

(4) Metzger, W. H. 1928 The effect of growing plants on solubility of soil nutrients. *Soil Sci.* 25: 273–280.

(5) Newton, J. D. 1924 Measurements of carbon dioxide evolved from the roots of various crop plants. *Sci. Agr.* 4: 268–274.

(6) Starkey, R. L. 1929 Some influences of the development of higher plants upon the microörganisms in the soil: I. Historical and introductory. *Soil Sci.* 27: 319–334.

(7) Starkey, R. L. 1929 Some influences of the development of higher plants upon the microörganisms in the soil: II. Influence of the stage of plant growth upon abundance of organisms. *Soil Sci.* 27: 355–378.

(8) Stoklasa, J. 1924 Die modernen Ziele der biochemischen Forschung des Bodens. *Chemie d. Zelle u. Gewebe*. 12: 23–44.

(9) Turpin, H. W. 1920 The carbon dioxide of the soil air. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 32: 319–362.

(10) Waksman, S. A. 1923 Microbiological analysis of soils as an index of soil fertility: V. Methods for the study of nitrification. *Soil. Sci.*, 15: 241–260.

(11) Waksman, S. A., and Starkey, R. L. 1924 Microbiological analysis of soils as an index of soil fertility: VII. Carbon dioxide evolution. *Soil Sci.* 17: 141–161.

IS SULFUR A LIMITING FACTOR OF CROP PRODUCTION IN SOME UTAH SOILS?[1]

J. E. GREAVES AND W. GARDNER[2]

Utah Agricultural Experiment Station

Received for publication February 27, 1929

Sulfur is an essential constituent of the bodies of plants and animals. Like nitrogen in its cycle, sulfur journeys through earth, air, and water, occurring in both the organic and the inorganic forms. Native nitrogen occurs in the atmosphere, native sulfur in soil and water. Sulfur and nitrogen find their way into the atmosphere through combustion and decay; they both are rendered available to the growing plants through biological processes. Animals require both nitrogen and sulfur in the organic form. A vast amount of work has been done on the nitrogen cycle, much less on the sulfur cycle. This is because of the different values which have been assigned to the two processes. Three modern discoveries, however, have given to the sulfur cycle a new interest: 1. Modern methods of analysis have shown that sulfur constitutes a greater proportion of plant and animal tissues than was formerly believed to be the case. 2. Soils have been found which contain insufficient sulfur to meet the needs of the growing plants. 3. It has been learned that sulfur may play an indirect rôle in the liberation of plant-food. With these facts in mind, a study has been made of: (*a*) The sulfur content of a limited area of the Cache Valley soils; (*b*) the sulfur removed from these soils by growing crops; (*c*) the quantity of sulfur brought to the soil by rain and irrigation waters; (*d*) the influence of sulfur carriers upon the bacterial activity of the soil, and (*e*) the influence of sulfur on the yield of barley.

SULFUR IN SOIL

Soils were collected from various localities in Cache Valley and analyzed for total sulfur. The samples were taken to a depth of 12 inches, great care being taken to see that a representative sample was obtained from each district. The total sulfur in the soil was determined by the moist-fusion method (2). The averages of closely agreeing determinations are given in table 1.

Two facts are evident from these results: First, there is a wide variation in the sulfur content of Cache Valley soils; second, the sulfur content of some of these soils is very low. This becomes evident in comparison with the sulfur

[1] Contribution from the department of bacteriology and chemistry, and department of physics. Publication authorized by director, February 21, 1929.

[2] It is a pleasure to acknowledge the kind assistance of C. T. Hirst, D. H. Nelson, J. Dudley Greaves, Lester Hirst, and Yeppa Lund in the analytical work.

content of soils from other parts of the United States. The soils of Missouri (5) were found to contain from 297 to 1067 pounds per acre-foot of soil. Iowa (3), 700 to 900; Oregon (16), 720 to 1200; Wisconsin (12), 660 to 2000; Nebraska (1), 828 to 2526; Kentucky (18), 900 to 2340; Kansas (20), 972 to 1656; Tennessee (15), 1850; and the average for the eastern part of the United States (17) is 1440 pounds per acre-foot of 3,600,000 pounds. These have all been calculated on the same bases as the Cache Valley soil, 3,600,000 pounds of soil per acre-foot. The sulfur content of the Greenville soil is lower than any of these, and all but two of the Cache Valley soils contain less sulfur than the average of the eastern part of the United States. The average for the Cache Valley soils is only 903 pounds per acre-foot. Oregon, Wisconsin, Iowa, and Missouri, all of which have been considered by some workers as deficient in sulfur, contain more sulfur than Cache Valley soils.

TABLE 1

Percentages and pounds per acre of sulfur in typical Cache Valley soils

LOCALITY	SULFUR	SULFUR IN 3,600,000 POUNDS SOIL
	per cent	*pounds*
Greenville Experiment Farm	0.007	252
Smithfield	0.014	504
Lewiston	0.019	684
Cache Junction	0.019	684
Avon	0.020	720
Trenton	0.023	828
Hyrum	0.024	864
Cornish	0.025	900
Richmond	0.033	1,188
Newton	0.043	1,548
Petersboro	0.049	1,764

A general estimation of the durability of a soil may be obtained by comparing the quantity of sulfur it contains plus its gain from various sources with the yearly sulfur loss. Table 2 indicates the quantity of sulfur removed yearly from the soil by maximum crops. In the case of wheat, oats, barley, and corn, the data have been obtained from average results obtained by the analyses of numerous samples grown under varying conditions in different sections of Utah (11). The sulfur content of the grains varied widely, depending upon variety, soil, and whether or not irrigation water was used. Therefore, under varying conditions, more or less sulfur may be removed by the same crop than is indicated by the figures given in table 2, but they may be taken as close approximations of what may be expected under general conditions. The sulfur in the other crops has been calculated from the work of Hart and Peterson (12). The determinations by these, as well as those by the present workers, were obtained by the moist-fusion method; consequently, they represent the

sulfur removed by the plants. This was not the case where the analyses were made on the ash.

It is evident that the sulfur removed annually from the soil varies widely with the crop. A 300-bushel crop of potatoes removes only 5 pounds, whereas an 8-ton crop of alfalfa removes 52.8 pounds. Moreover, it has been shown elsewhere (11) that there may be a great variation in the sulfur content of grain; for example, there was found to be a difference of 230 per cent in the sulfur content of wheat, 255 per cent in the case of oats, and 293 per cent in the case of barley, depending upon whether much or little was available to the growing plant.

TABLE 2

Pounds of sulfur removed from an acre of soil by maximum crops

CROPS	SULFUR
	pounds
Corn grain (100 bu.)	9.5
Corn stover (3 tons)	7.6
Total crops	17.1
Barley grain (100 bu.)	7.4
Barley straw (2.5 tons)	7.4
Total crops	14.8
Oats grain (100 bu.)	6.1
Oat straw (2.5 tons)	6.0
Total crops	12.1
Alfalfa (8 tons)	52.8
Peas (4 tons)	13.6
Sugar beets (20 tons)	13.0
Potatoes (300 bu.)	5.0

The exact nature of the process of assimilation of sulfur or other plant-food substance from the soil is not known. It may perhaps be assumed, however, without serious error that the rate of increase of crop yield with increase in sulfur is proportional to the magnitude of the sulfur deficiency in the soil. In order to obtain a first approximation as to the nature of the influence of sulfur on crop yield, it is doubtless appropriate also to assume that the time rate of depletion of sulfur (on the supposition that none is added from outside sources) is proportional to the product of the sulfur content of the soil and the crop yield. These two hypotheses lead to the conclusion that the crop yield will diminish from year to year at a rate dependent upon the nature of the physiological requirement of the plant for this substance as a plant-food and upon the length of time sulfur-requiring plants have been grown upon the soil.

In order that this may be brought out somewhat more quantitatively, the

mathematical development leading to an exponential relationship is here presented in full.

If we introduce the symbols:

y = crop yield per annum,
s = sulfur content of soil,
s_0 = sulfur content of soil required for optimum growth,
k_1 and k_2 represent proportionality constants as yet undetermined,
t = time,

we may set down the following equations expressing the hypotheses named above:

$$dy/ds = k_1\,(s_0 - s) \tag{A}$$
$$ds/dt = -k_2\,(sy) \tag{B}$$

Integrating (A), we obtain

$$y = k_1\,(s_0 s - s^2/2) + k_3. \tag{C}$$

Differentiating this equation with respect to the time,

$$dy/dt = k_1\,(s_0 - s)\,ds/dt. \tag{D}$$

Eliminating ds/dt by means of (B), equation (D) becomes

$$dy/dt = -c(s_0 - s)\,sy. \tag{E}$$

where c is defined as

$$c = k_1k_2.$$

Upon integration equation (E) becomes

$$y = y_0e^{-c\int(s_0 - s)s\,dt} \tag{F}$$

It is to be noted that the exponent will approach a finite limiting value as s approaches zero, and if the integration constant of equation (c), k_3, is taken as zero, we may conclude that y will approach zero asymptotically. Equation (E) indicates also that the tangent to the curve of equation (F) will be zero when s is at the optimum value s_0, so that we may infer without any attempt to complete the integration of equation (F) that the relationship between y and t will conform to some such family of curves as is represented in figure 1.

As has been shown (4) in connection with a study of the economic application of irrigation water, the sulfur content of the soil should not be allowed to fall below the point determined by the following equation,

$$k_1\,(s_0 - s) = e/a \tag{G}$$

where e represents the cost per unit quantity of sulfur applied and a the value of unit quantity of crop produced. The question, therefore, as to whether

or not sulfur should be added as a fertilizer will depend upon the constant k_1. which will be characteristic of the crop and of the soil, and upon the constants e and a, which will depend upon the locality. The comparatively low content of sulfur in Cache Valley soils and the small amount in the irrigation streams supplying water for parts of the valley indicate that the problem is deserving of careful attention.

SOIL GAINS IN SULFUR

Combustion and decay are constantly liberating gaseous sulfur compounds. Millions of tons of sulfur are thrown into the atmosphere by volcanoes and from chimneys, especially from some manufacturing plants. This sulfur finds

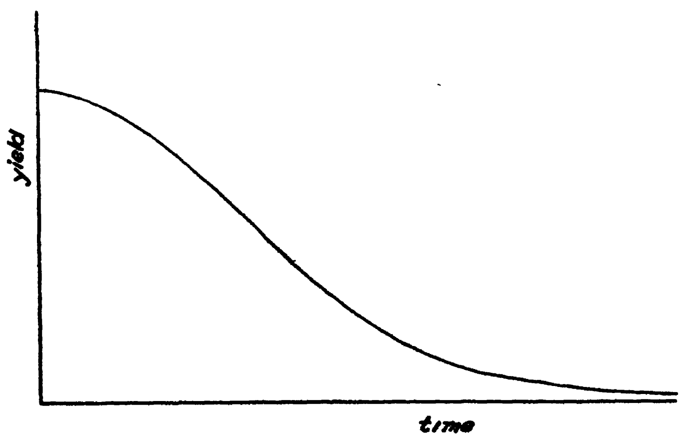


FIG. 1. CURVE ILLUSTRATING DECREASE IN CROP YIELD WITH TIME, AS SULFUR DIMINISHES, AS SHOWN BY EQUATION F

its way back into the oceans, lakes, and streams of the country, and into the soil. The quantity of sulfate discharged into the sea is enormous. The salts of its waters contain about 7.69 per cent of the sulfate radical; consequently, one would expect some sulfur brought to irrigated soils by irrigation waters. The principal irrigation waters of Utah have been analyzed, and in table 3 is indicated the pounds of sulfur brought to the soil by one acre-foot of the specific waters. These results are the averages of many determinations made during different years and seasons of the year; consequently, they represent rather accurately the sulfur which soils may be expected to receive with irrigation waters.

Emery Creek contains 676 pounds of sulfur in an acre-foot of water, Price River, Huntington Creek, Sevier River, Jordan River, and Ferron River all carry over 200 pounds of sulfur in an acre-foot of water. Consequently, none of the lands irrigated with these waters would become deficient in sulfur. These waters are high in alkali salt, however, and because of their high salt

content (10) may be injurious. The water of five streams and one lake tested contained between 100 and 200 pounds of sulfur in an acre-foot of water; seven others carried between 50 and 100 pounds. Twenty-seven streams carried less than 50 pounds, and all but two of these carried less than 25 pounds of sulfur in an acre-foot of water. Consequently, very appreciable quantities of sulfur are carried to the soil by some irrigation waters, and the quantity varies greatly with the streams. Some waters would furnish sufficient for even maximum irrigation crops of even heavy sulfur-requiring plants, whereas

TABLE 3

Amount of sulfur contained in one acre-foot of water from various sources

STREAM	SULFUR PER ACRE-FOOT WATER	STREAM	SULFUR PER ACRE-FOOT WATER
	pounds		*pounds*
Emery Creek	676	Spring Creek	36
Price River	574	Salt Creek	33
Huntington Creek	343	Parley's Creek	37
Sevier River	228	Big Cottonwood	31
Jordan River	205	Provo River	31
Ferron River	203	West Cache Canal	30
Utah Lake	198	Hobble Creek	24
Beaver River	189	Rock Creek	23
Uinta River	184	Weber River	19
Lake Fork	178	Little Cottonwood	19
Green River	165	Parowan Creek	16
Cottonwood	124	North Creek	15
Mill Creek	91	Summit Creek	14
Strawberry	87	Indian Creek	12
Cedar Creek	78	Clear Creek	11
Bear River	76	Ogden River	11
Santa Clara	60	Little Bear River	9
American Fork	54	Box Elder Creek	9
Emigration Creek	50	Red Creek	8
Spanish Fork	49	Summit Creek	7
Ashley	44	Logan River	7
Duchesne	40	Maple Creek	7
Beaver	38	Cub River	3

other waters would carry insufficient quantities but would assist in maintaining the sulfur content of the soil. It is interesting to note that the principal streams of the Cache Valley region, Cub River, Logan River, and Little Bear River carry only small quantities of sulfur. This raises the question: Will soils watered and especially drained by other streams low in sulfur likewise be associated with soils deficient in sulfur? The likelihood is great that this will be the case. The Cache Valley soils irrigated from West Cache Canal and Bear River would receive appreciable quantities of sulfur in the irrigation waters, but the soils watered by the other streams would not.

In addition to the sulfur brought to the soil in irrigation water, there is an annual gain from the atmosphere, brought in by the precipitation. During the years 1924 to 1927, inclusive, samples of rain and snow from the rain gages of the valley were collected after each storm, stored in glass-stoppered bottles, and analyzed twice a year. Samples were collected from ten localities in Cache Valley. The average annual pounds per acre brought to the soil in rain and snow are indicated in table 4.

The high results obtained on the college campus are undoubtedly due to the large quantities of soft sulfur-bearing coal being burned near the rain gauge where the precipitation is collected. The sulfur brought annually to the soil per acre in the other localities varied from 6.4 to 12.1 pounds of sulfur, with an average of 9.5 pounds in the valley. This being an average from nine stations

TABLE 4

Pounds per acre of sulfur carried to soil annually by precipitation

LOCALITY	ANNUAL AMOUNT OF SULFUR PER ACRE
	pounds
College Hill	32.7
Newton	12.1
Hyrum	11.6
Petersboro	10.9
Richmond	10.7
Avon	9.3
Cache Junction	9.1
Lewiston	8.7
Smithfield	6.6
Cornish	6.4

and extending over a period of four years makes it probable that it represents approximately the quantity of sulfur these soils receive annually in the precipitation. An examination of the results reported by Joffe (13) of the sulfur brought to the soil in other places reveals the fact that the results at the Utah station are far lower than those reported for Garforth, England; Leeds, England; Petrograd, Russia; Urbana, Illinois; Ithaca, New York; Tennessee; and Mt. Vernon, Iowa, where the annual amount of sulfur brought to the soil per acre varies from 26 to 131 pounds. These places are located near centers where large quantities of coal are burned; consequently they receive greater quantities of sulfur than would soil in the open country.

The quantity of sulfur brought to the soil in some parts of Russia, Sicily, New Zealand, Rothamsted (England), Samaria, and Wisconsin are nearer the quantity of 6 to 10 pounds per acre brought annually to the Cache Valley soils.

If we assume, therefore, that the sulfur brought to Cache Valley soils annually in the precipitation is 9.5 pounds per acre and 7 pounds per acre annually in the irrigation water (the actual quantity found in the Logan River

water which is used on some of these soils), there would be an annual gain of 16.5 pounds. Where the Cub River water is used on these soils there would be an annual gain of only 12.5 pounds. Where barnyard manure is used there would be sulfur added with the manure. It is likely that the sulfur brought to the soil annually by precipitation and irrigation water would be about sufficient to account for the annual removal in such crops as wheat, oats, barley, corn, peas, and sugar beets. Where crops like alfalfa are grown, the sulfur of the rain and irrigation water would be far from sufficient to meet the needs of the growing plants. Consequently, from these results it may be concluded that with some crops sulfur may become a limiting element in some of the Cache Valley soils, and that at a not-far-distant date.

In all of the preceding calculations no account has been taken of the sulfur lost in drain waters, nor do we have any data on this phase of the subject. The data compiled by Joffe (13), however, show an annual loss of from 8 to 281 pounds of sulfur removed in the drainage water. The quantity lost would vary with the kind of soil, the amount of precipitation, the system of cropping, and the methods of cultivation.

It has been found by Jones (14) that the annual loss of sulfur from drainage in some of the sulfur-poor soils of Oregon, is 15 pounds. Now, if we accept this as an approximation of the loss from the Cache Valley soils and that annually there are 7 pounds brought to the Greenville soil per acre in the irrigation waters and 9.5 pounds in the precipitation, there would remain in the soil 1.5 pounds per acre annually over that removed to supplement the native soil sulfur. Even with crops requiring only small quantities of sulfur this would not meet their need, and with some other crops—alfalfa, for instance—it would be an insignificant quantity. Consequently, these results point to the conclusion that some of the Cache Valley soils will ultimately become deficient in sulfur. The time required for this to become evident will vary with the specific soil, the nature and the size of the crop grown, and the quantity of manure returned to the soil. This is just the opposite of the conclusion reached by Stewart (19) who figures on an annual gain of 45 pounds per acre, which is more comparable to the figure obtained for the precipitation on the college campus. Using the figure, 35 pounds annually, one could conclude with Stewart that sulfur is not a limiting element in crop production.

INFLUENCE OF SULFUR ON SOIL BACTERIA

The small quantity of sulfur in the Greenville soil made it appear probable that, when added to the soil, sulfur would increase its bacterial activities. This was tested by Fife (6) who added sulfur to soils and found that for the short time under observation sulfur had no uniform effect upon the number of bacteria and upon the nitrogen-fixing powers of the soil but that ammonification was increased from 50 to over 100 per cent, depending upon the soil and the amount of sulfur applied. In some cases the nitrification was increased over 100 per cent. These effects could have come from the rapid oxidation

of the sulfur with the production of sulfuric acid, which would increase the available plant nutrients in the soil. For this reason, the influence of sulfur-carrying salts upon the ammonifying, nitrifying, and nitrogen-fixing powers

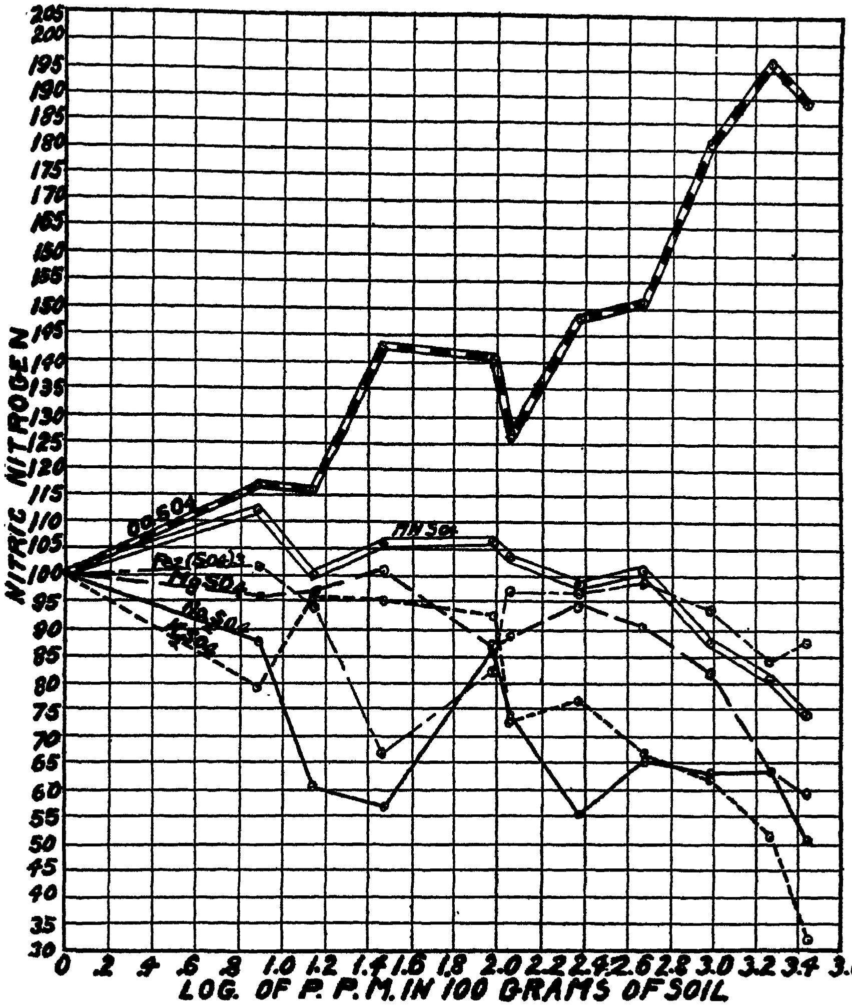


FIG. 2. ILLUSTRATING AMMONIA ACCUMULATION IN SOIL TO WHICH HAVE BEEN ADDED VARYING AMOUNTS AND KINDS OF SULFUR-CARRYING SALTS

of the soil has been determined. The determinations were made by the tumbler method on Greenville soil to which various quantities of the sulfur carriers were added. In the ammonifying and nitrifying tests one per cent of dried blood was added, whereas in the nitrogen-fixation tests one per cent of lactose was added to the soil. The sulfates of sodium, potassium, calcium, magne-

sium, iron, and manganese were used. The average results are given in figure 2. The concentrations of the various salts are stated as the logarithm of parts

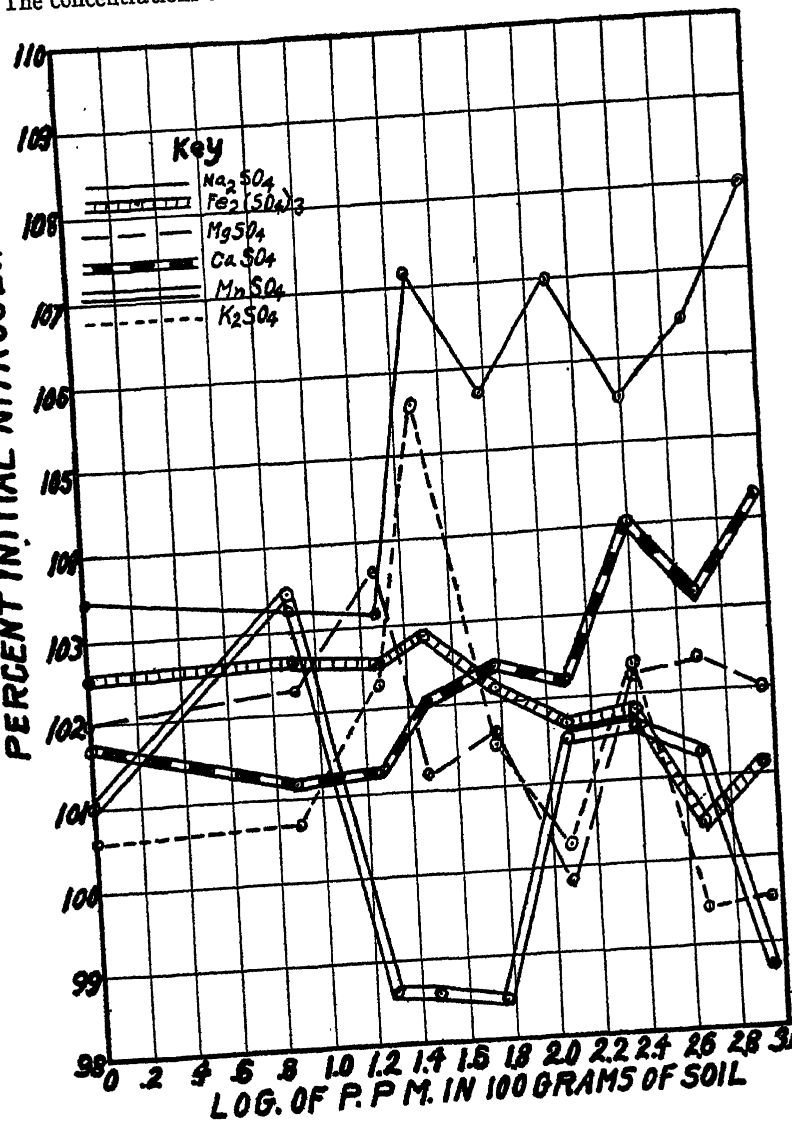


FIG. 3. ILLUSTRATING NITRATE ACCUMULATION IN SOIL TO WHICH HAS BEEN ADDED VARYING AMOUNTS AND KINDS OF SULFUR-CARRYING SALTS

per million of the sulfate ion in 100 gm. of soil. The untreated soil is taken as producing 100 per cent of ammonia. There is a stimulation due to the

manganese, calcium, and iron sulfate but not to the sodium, potassium, and magnesium sulfates, which would lead to the conclusion that the action is probably due to the cation and not the anion.

The results for nitrification are given in figure 3. Both manganese and calcium sulfate stimulate, the latter to a marked extent, but it is likely that the stimulation is due to an indirect action of the salt (9).

The results for nitrogen fixation are given in figure 4. All the sulfates increase nitrogen fixation to a varying degree, depending upon the specific sulfate and the quantity added. In pot experiments extending over a series

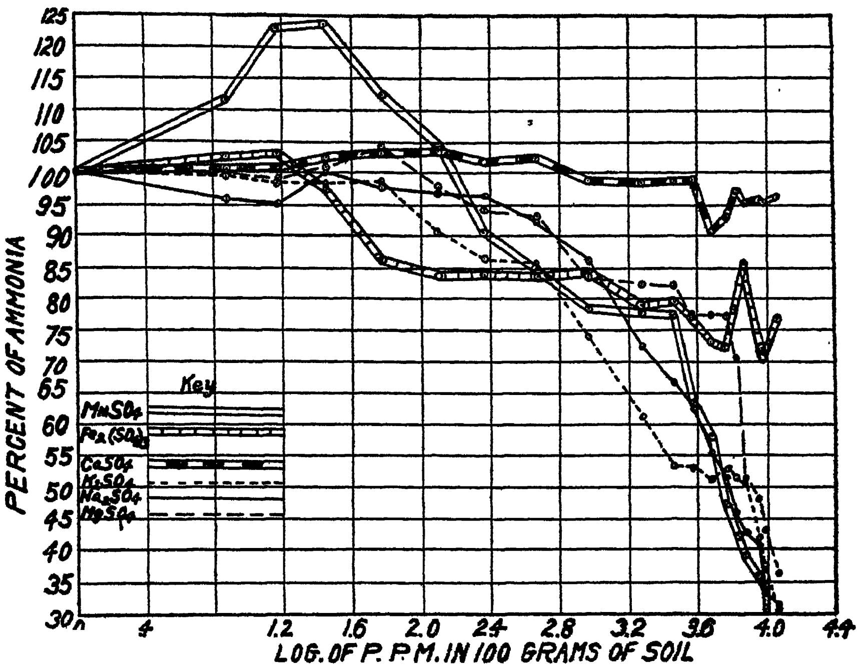


FIG. 4. ILLUSTRATING SOIL GAINS IN NITROGEN WHERE VARYING QUANTITIES AND KINDS OF SULFUR-CARRYING SALTS ARE APPLIED TO THE SOIL

of years it has been shown that the increase in nitrogen is often considerable (8), and it is only in the case of sulfate-treated soils that the typical azotobacter membrane was observed (7). Up to the present, however, pot experiments with barley as the crop have given no regular results from which it might be concluded that sulfur is or is not beneficial.

SUMMARY

An analysis of the Cache Valley soils shows them to contain from 252 to 1764 pounds of sulfur per acre-foot of 3,600,000 pounds. The average sulfur content of the soil analyzed was 903 pounds per acre-foot.

On the basis of certain simplifying assumptions, a mathematical equation is developed indicating the general form of the relationship between the crop yield and the time where no sulfur is added from outside sources.

On the basis of this equation and by means of the law of diminishing returns, a method of determining the critical sulfur content in terms of soil and crop characteristics and economic constants is illustrated.

The quantity of sulfur carried from the soil varies with the crop, the quantity of water applied and the composition of the soil.

Analyses of the water of 45 streams, the waters of which are used for irrigation purposes, showed them to carry from 3 to 676 pounds of sulfur per acre-foot of water. Six streams carried over 200 pounds and 34 streams carried less than 100 pounds. Consequently, the quantity of sulfur carried to the soil by irrigation water is often appreciable. The waters used on the soils of Cache Valley contain only small quantities of sulfur.

The precipitation was collected in ten different localities in the valley. The average annual quantity of sulfur brought to the soil over a period of 4 years at nine of the ten stations varied from 6.4 to 12.1 pounds, with an average of 9.5 pounds. The precipitation collected near the college campus had an average annual sulfur content of 32.7 pounds.

Sulfur-carrying salts increase the bacterial activities of the soil. This is especially pronounced in the case of nitrogen fixation. This may be due either to the direct action of the sulfur as a food, to the microörganisms, or to an indirect action upon insoluble nutrients.

The conclusion is reached that sulfur may become a limiting factor of crop production in some Cache Valley soils. The time required for this to manifest itself in diminished crop returns will vary with the soil, the specific irrigation water used, and the crop grown upon the soil.

REFERENCES

(1) Alway, J. F., and Rost, C. C. 1916 The loess soils of the Nebraska portion of the transition region: IV. *Soil Sci.* 1: 405–436.

(2) Association of Official Agricultural Chemists 1925 Official and Tentative Methods of Analysis, p. 30, 31. Assoc. Off. Agr. Chem., Washington, D. C.

(3) Brown, P. E., and Kellogg, E. H. 1914 Sulfofication in soils. Iowa Agr. Exp. Sta. Res. Bul. 18.

(4) Clyde, H. S., Gardner, W., and Israelsen, O. W. 1923 The economical use of irrigation water based on tests. *Engin. News. Rec.* 91: 548–552.

(5) Duley, F. L. 1916 The relation of sulfur to soil productivity. *Jour. Amer. Soc. Agron.* 8: 154–160.

(6) Fife, J. M. 1926 The effect of sulfur on the microflora of the soil. *Soil. Sci.* 21: 245–252.

(7) Greaves, J. E. 1926 The microflora and productivity of leached and non-leached alkali soil. *Soil Sci.* 3: 271–302.

(8) Greaves, J. E. 1927 The influence of soluble salts and organic manures on soil nitrogen. Proceedings and Papers. *First Internatl. Cong. Soil Sci.* 3: 213–221.

(9) Greaves, J. E., and Carter, E. G. 1919 The action of some common soil amendments. *Soil Sci.* 7: 121–160.

(10) GREAVES, J. E., AND HIRST, C. T. 1918 Composition of the irrigation waters of Utah. Utah Agr. Exp. Sta. Bul. 163.

(11) GREAVES, J. E., AND HIRST, C. T. 1928 The mineral content of grains. *Jour. Nutrition* 1: 293–298.

(12) HART, E. B., AND PETERSON, W. H. 1922 Sulfur requirements of farm crops in relation to soil and air supply. Wis. Agr. Exp. Sta. Res. Bul. 14.

(13) JOFFE, J. S. 1922 Biochemical oxidation of sulfur and its significance to agriculture. N. J. Agr. Exp. Sta. Bul. 374.

(14) JONES, J. S. 1928 The ratio of sulfur to phosphorus in western Oregon soils and losses of sulfur through drainage and cropping. *Soil Sci.* 26: 447–453.

(15) MACINTIRE, W. H., WILLIS, L. G., AND HOLDING, W. H. 1917 The divergent effect of lime and magnesia upon the conservation of soil sulfur. *Soil Sci.* 4: 231–237.

(16) REIMER, F. C., AND TARTAR, H. V. 1919 Sulfur as a fertilizer for alfalfa in southern Oregon. Ore. Agr. Exp. Sta. Bul. 163.

(17) ROBINSON, W. O. 1914 The inorganic composition of some important American soils. U. S. Dept. Agr. Bul. 122.

(18) SHEDD, O. M. 1913 The sulfur content of some typical Kentucky soils. Ky. Agr. Exp. Sta. Bul. 174.

(19) STEWART, R. 1920. Sulfur in relation to soil fertility. Ill. Agr. Exp. Sta. Bul. 227.

(20) SWANSON, C. O., AND MILLER, R. W. 1917 The sulfur content of some typical Kansas soils and the loss of sulfur due to cultivation. *Soil Sci.* 3: 129–148.

NITRATES IN SOIL AND PLANT AS INDEXES OF THE NITROGEN NEEDS OF A GROWING CROP[1]

B. E. GILBERT AND J. B. SMITH

Rhode Island Agricultural Experiment Station

Received for publication March 5, 1929

The Rhode Island Agricultural Experiment Station has been engaged for some time in a series of attempts to determine the feasibility of controlling optimum fertilization of market-garden and field crops by the maintenance of optimum concentrations of mineral nutrients in the plant solution or in the soil extract, as measured by chemical analyses. Two papers have appeared, suggesting tentative minimum concentrations to be exceeded to insure normal yields. In 1927 "The current mineral nutrient content of the plant solution as a possible means of chemical control of optimum fertilization" was discussed by Gilbert and Hardin (2), and in 1928 Smith (7) made suggestions concerning "Desirable soil-nitrate levels for certain market-garden crops."

During the growing season of 1928 further efforts were made to control soil and plant-nitrate levels by side-dressing with soluble nitrogen fertilizers at intervals during the season. This paper is concerned with two aspects of the problem:

1. The 1928 attempts to control nitrate concentrations.
2. A comparison of the soil and plant as sampling sources with regard to their general applicability for the prediction of a future nitrogen need of a crop.

CHEMICAL METHODS

The methods of measuring nitrates have been described in detail elsewhere. The methods used in measuring the nitrate nitrogen in the soil were those of the junior author (7), and determinations of nitrates in the plant solution were made according to the technique described by the senior author (1). The analyses here reported are in terms of nitrate nitrogen in the expressed plant solution and that in oven-dry soil.[2]

GROWTH MEASUREMENTS

It has been realized that in order to approach more definitely any problem which involves the study of modifications in yields some knowledge of the growth curve for each crop is essential. Therefore, study has been given to the problem of how best to measure growth. It was early recognized that the

[1] Contribution No. 378 of the Rhode Island Agricultural Experiment Station.

[2] The plant solution nitrate determinations were made by Donald E. Frear, and J. Eric Blaney assisted in the work with soil.

ideal method must be simple. Actual measurements of cabbage were tried in 1926 and reported by Gilbert, et al. (3), but this method proved too time-consuming and had too high a labor cost for the results obtained.

In 1928 a weekly photographic record of celery, lettuce, and cabbage was taken. In order to obtain comparable records the focal distance must be kept the same from week to week. This was secured by suspending the camera from an extension bolted to the top of a stepladder. By staking off the position of the stepladder and taking the pictures at the same hour, weekly records were obtained. The areas covered by each individual in the cases of cabbage and

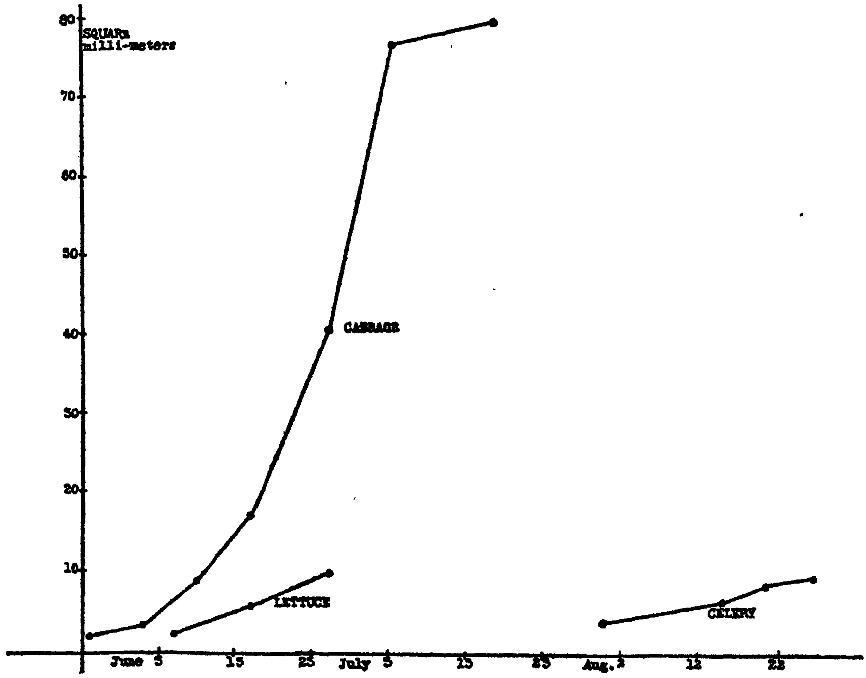


Fig. 1. Growth Curves Based on Photographic Measurements

lettuce, and the row areas with celery were computed by means of caliper measurements made on the photographs. A single plant average per plat was thus secured. These figures were further averaged between plats differing in nitrogen treatments. By this means, weekly points on a curve of growth were obtained (fig. 1). It is recognized that this method of measurement is somewhat rough but it is doubted whether the time and labor involved in the use of a planimeter would justify any small increases in accuracy which might result.

To date this method of measuring growth has proved feasible only with crops which have a horizontal habit of growth. It was tried with corn, but

the results did not warrant further use. As in all experimental work, the number of individuals photographed largely determines the error of the method. Thus, for celery the growth curve can be considered more accurate than for cabbage where only four individuals could be photographed.

AGRONOMICAL DATA

The crops studied were early cabbage, early tomatoes, fall celery, and fall beets. These crops were grown on a permanent 3-year market-garden rotation established in 1916 and described by Hartwell and Crandall (5). Further history of this rotation is given by Smith (7). Table 1 gives a summary of the fertilization during 1928, and also the yields obtained. It will be noted that

TABLE 1

Total fertilization and yields of 1928 market-garden crops

CROP	TREATMENT	CHEMICALS			MANURE-COMPOST	YIELD PER ACRE
		N	P_2O_5	K_2O		
		pounds per acre	*pounds per acre*	*pounds per acre*	*tons per acre*	
Cabbage	Standard	120	100	90	....*	465 barrels
	Low N	60	100	90	....*	423 barrels
Tomatoes	Standard	90	120	90	....†	328 bushels
	Low N	30	120	90	....†	289 bushels
Celery	Standard	90	120	50	26	375 hundredweight
	Low N	30	120	50	26	315 hundredweight
Beets	Standard	75	100	50	....*	335 bushels
	Low N	24	100	50	....*	250 bushels

* Thirty-two tons in each 3-year round; none the cabbage-beet year.

† Green manure annually.

the sole variant was the nitrogen and that a definite increase in yield was obtained where the larger quantities of nitrogen were used. Although a large proportion of the fertilizer nitrogen was ammonia nitrogen, a rapid rate of nitrification usually left only traces of ammonia in the soil, as was shown by ammonia determinations made by Harper's method (4) in soil samples taken weekly.

Early cabbage

Golden Acre cabbage plants were set April 19. The harvest began July 9 and was completed on July 26. The initial fertilizer (table 1) application was made just before the plants were set. No manure was used in the current year, but these plats received a total of 32 tons of manure-compost in each 3-year round of the rotation. The yields were somewhat higher than the 11-year average reported by Hartwell and Crandall (6)

Tomatoes

Murry's New Early Wonder tomato plants were set May 23. The harvest began on July 20 and finished September 7. The tomato crop, as described in this paper, was grown on plats which had been green-manured with buckwheat the previous fall. Although the vine growth was very vigorous, the yields of fruit were somewhat depressed, possibly because of adverse weather conditions which influenced the pollination and thus the set of fruit. Wind injury to the foliage in mid-July may have further reduced the yields.

TABLE 2

Applications of nitrogen during the growing season of 1928

CROP	TREATMENT	INITIAL FERTILIZATION		DATE	SIDE-DRESSINGS		TOTAL N PER ACRE
		$NaNO_3$—N per acre	$(NH_4)_2SO_4$—N per acre		$NaNO_3$—N per acre	$(NH_4)_2SO_4$—N per acre	
		pounds	*pounds*		*pounds*	*pounds*	*pounds*
Cabbage	Standard	9	19	May 17	9	19	28
	Standard	....	....	June 5	9	19	28
	Standard	....	....	June 28	8	17	25
	Low N	5	9	May 17	5	9	14
	Low N	....	....	June 5	6	11	17
Tomatoes	Standard	7	19	July 6	9	19	28
	Standard	....	....	July 25	9	13	22
	Low N	2	5	July 6	3	5	8
Celery	Standard	0	0	August 10	9	19	28
	Standard	....	....	August 31	9	19	28
	Standard	....	....	September 21	12	23	35
	Low N	0	0	August 10	5	6	11
	Low N	....	....	August 31	5	14	19
Beets	Standard	9	19	September 21	9	19	28
	Standard	....	....	October 3	7	13	20
	Low N	4	8	September 21	4	8	12

Celery

Golden Plume celery plants were set July 12, and cut about the middle of October after being boarded during the last week of September

This crop was grown on a stable-manure rotation and the yields were as shown in table 1. These yields, which were exceptionally good as compared with average yields of former years, coupled with growth observations, show that the celery crop was supernormal. This may have been caused by the

rather exceptional moisture conditions during the growth period of celery, brought about by a providential rain at setting time and unusually high precipitation during September and October.

Fall beets

A mixture of Crosby's Egyptian and Early Wonder seed was sown July 31. The beets were pulled late in October. The plats concerned were fertilized following the removal of the early cabbage crop (table 2). The yields obtained were considered to be normal.

CHEMICAL CONTROL

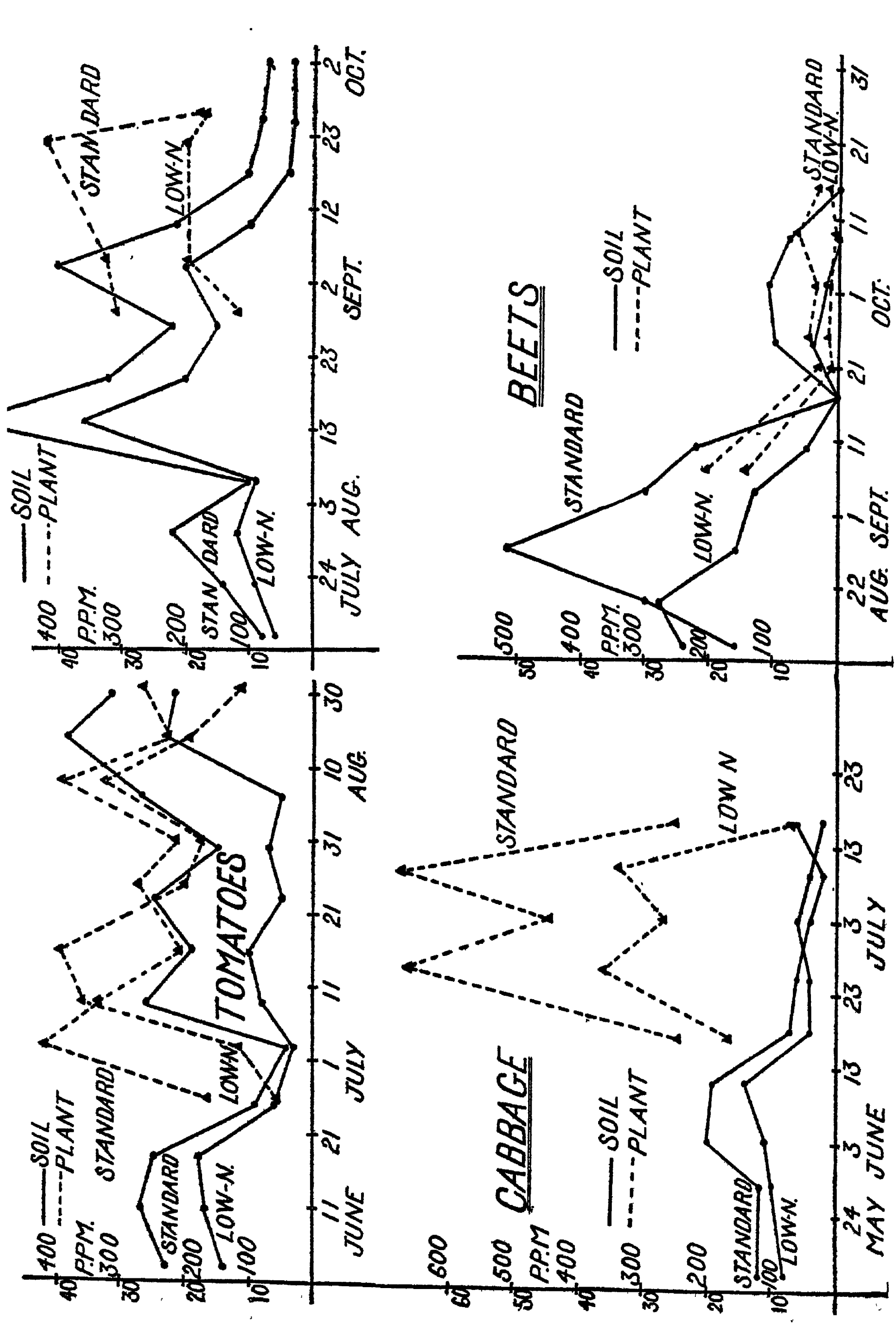
During 1928 an attempt was made to maintain definite levels of nitrogen feeding by applying portions of the nitrogen fertilizer as side-dressings whenever the soil-nitrate determinations indicated them to be necessary. The amounts and dates of applications of side-dressings are given in table 2. Some idea of the effectiveness of this method may be gathered from the graphical description of soil and plant nitrates shown in figure 2. Rapid fluctuations in metabolism in the plant, microbiological action, and nitrate movement in the soil made it impractical and perhaps impossible to control nitrate concentrations except within wide limits. With cabbage, tomatoes, and celery the nitrates in both plant solution and soil were maintained for the plat receiving optimum fertilizer at, or above, the levels recommended by Smith and Gilbert for the greater part of the season. With beets, however, the concentrations were allowed to drop before side-dressings were applied, after which time it seemed difficult to raise the concentrations to those recommended.

Early cabbage

The maximum growth period of cabbage was during the month of June, as is shown by the curve of growth in figure 1. Side-dressings applied May 17 and June 5 influenced the soil-nitrate curve whereas that applied June 28 was reflected by the plant-solution nitrate alone. The depression in the soil-nitrate curve from June 11 forward is of especial interest in the light of high nitrates in the plant during this period. Doubtless the demand of the plant was so great as to rapidly deplete the soil of nitrates. The cabbage crop was in the heading stage during the latter portion of this period and this, without doubt, accounted for the very rapid depletion of the soil even after the extra application of fertilizer nitrogen on July 28. The optimum fertilizer plat had soil-nitrate concentrations above 10 p.p.m. until June 18. For the rest of the season the nitrate nitrogen in plant solution was above the recommended 300 p.p.m.

Tomatoes

The side-dressing of nitrogen which was applied July 6 produced increases in both soil and solution nitrates, whereas that on July 25 did not influence the determinations made on July 30. This is doubtless explained by the fact


TOMATOES
CABBAGE
BEETS
SOIL
PLANT
STANDARD
LOW-N.
P.P.M.
JUNE
JULY
AUG.
SEPT.
OCT.
MAY

that no rain fell between July 25 and July 30, and the fertilizer had not penetrated the soil. This application appeared in later determinations, as shown in the tomato curve (fig. 2). The nitrate nitrogen of the soil was maintained quite consistently above 20 p.p.m. and that of the solution above 300 p.p.m. for the entire season on the optimum plat (fig. 2).

Celery

The influence of applications of fertilizer on August 10 and August 31 is indicated by the soil-nitrate curve for celery (fig. 2).

The growth curve (fig. 1) of celery shows that there was no very pronounced maximum period during which the celery drew excessively on soil nitrates. Thus the maintenance of levels with this crop should be easier than with a rapidly growing crop such as spring cabbage. Low temperature conditions, during the time when celery would naturally make its greatest demand for nitrogen, were doubtless a factor, since this would slow down metabolism and thus limit the absorption of soil nitrates.

Concentrations of nitrate nitrogen were maintained above the recommended 10 p.p.m. in soil and 300 p.p.m. in plant solution for the greater portion of the season.

Fall beets

With beets, side-dressings were not applied until the soil nitrates were reduced to a trace. The application made on September 21 affected both the soil and solution nitrates, whereas that of October 3 was apparently quickly secured by the plant and, therefore, shows only in the solution curve. Because of the late date at which the first side-dressing was applied, the nitrates in both soil and solution were much below the desired levels for a considerable portion of the growth period.

Except in the case of tomatoes, the optimum fertilizer plats produced crops comparable in yield to the 11-year average of Hartwell and Crandall (6). With beets as an exception, optimum nitrogen fertilizer was correlated with the recommended levels of nitrates in soil and solution and also with higher yields. Beets gave greater yields with the optimum fertilizer than with less nitrogen.

EVALUATION OF METHODS

It is of interest to note that although two methods were used in this attempted control of fertilization, neither of them can be considered to conform perfectly to the demands upon them. An ideal method should, with ease and speed of manipulation, provide accurate indications of the need for further fertilization at any given point in the growth of a crop. A study of the soil-nitrate curves shows that, whereas in the early stages of growth when the demands of the crop were small, the method served to portray accurately the nitrate reserves available to the crop, later in the season the picture was not always clear.

To demonstrate this, curves for the averages of both the soil nitrates and the

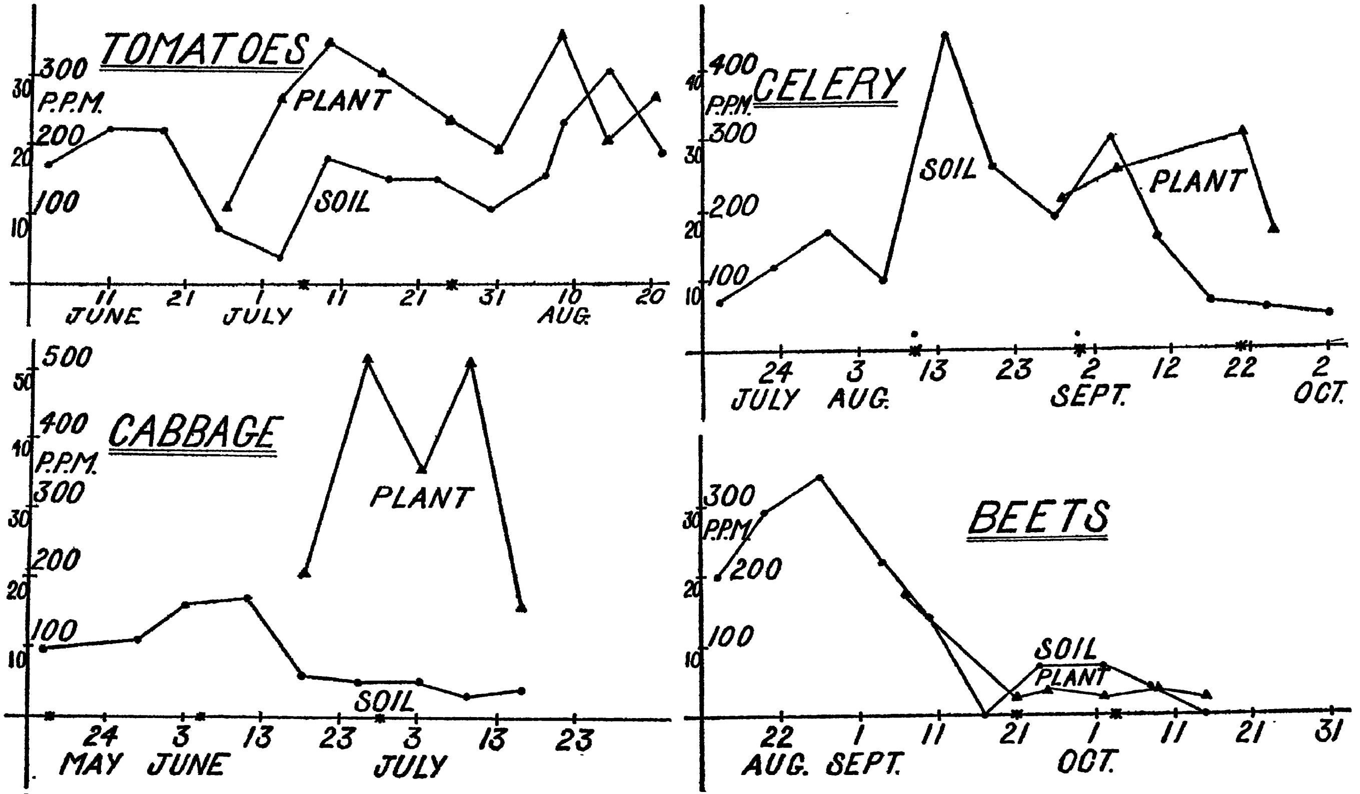


FIG. 3. COMPARISONS OF AVERAGE CURVES OF NITRATE NITROGEN IN SOIL AND PLANTS THROUGHOUT THE 1928 GROWING SEASON

Soil nitrates are indicated on the ordinates by numbers from 0–60 and plant solution nitrates by numbers from 0–600. Dates of fertilize de-dressings are indicated by stars on the abscissas.

plant-solution nitrates are presented (fig. 3). For tomatoes and beets the curves for soil and crop are nicely correlated but it is evident that the incipient nitrogen starvation, predicted by the low soil nitrates under rapidly growing cabbage and celery late in the season, was contradicted by the abundant nitrate reserve found in the plant. Had reliance been placed on soil conditions alone, nitrogen would have been used in excess of actual needs, and possibly to the detriment of quality in the crop. The soil method insures against nitrogen starvation but does not protect from over-feeding.

Analysis of the plant solution brings the chemist closer to the scene of metabolism and depicts current conditions much more sharply than can a study of the soil. Its disadvantages lie in a more lengthy manipulation and in difficulty in securing sufficient plant material when the crop is small. When the needs justify the labor, and frequent sampling is possible, this method should prove the better of the two.

For the control of nitrogen metabolism a combination of both methods should be superior to either one alone

SUMMARY

In this paper two aspects of the control of fertilization of market-garden crops are discussed.

1. The curves for soil and plant solution nitrates in connection with 1928 crops of cabbage, tomatoes, celery, and beets are given and attention is drawn to the influences of side-dressings of soluble nitrogen fertilizer upon these curves. Nitrate concentrations both in soil and in plant solutions were maintained above previously designated suboptimum concentrations throughout the greater part of the season and yields were uniformly greater than with lower nitrogen fertilization.
2. Control of nitrate concentrations within narrow limits proved impossible.
3. A comparison of the chemical methods used as to their usefulness as indexes of fertilizer need is made. The conclusion is drawn from the data that both methods should be used concurrently in order to secure a complete picture of nitrogen needs.
4. The determination of soil nitrates predicts the nitrogen needs of young plants adequately, but plant solution analyses are more exact for later growth stages.

REFERENCES

(1) GILBERT, B. E. 1926 The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates, and potassium in plant solutions. *Plant Physiol.* 1: 191–199.

(2) GILBERT, B. E., AND HARDIN, L. J. 1927 The current mineral nutrient content of the plant solution as a possible means of chemical control of optimum fertilization. *Jour. Agr. Res.* 35: 185–192.

(3) GILBERT, B. E., MCLEAN, F. T., AND ADAMS, W. L. 1927 The current mineral nutrient content of the plant solution as an index of metabolic limiting conditions. *Plant Physiol.* 2: 139–151.

(4) HARPER, H. J. 1924 The determination of ammonia in soils. *Soil Sci.* 18: 409–418.
(5) HARTWELL, B. L., AND CRANDALL, F. K. 1925 The substitution of stable manure by fertilizers, green manures, and peat: II. R. I. Agr. Exp. Sta. Bul. 201.
(6) HARTWELL, B. L., AND CRANDALL, F. K. 1928 The substitution of stable manure by fertilizers, green manures, and peat: III. R. I. Agr. Exp. Sta. Bul. 216.
(7) SMITH, J. B. 1928 Desirable soil-nitrate levels for certain market-garden crops. *Soil Sci.* 26: 265–279.

THE COMPARATIVE ACID TOLERANCE OF SOME SOUTHERN LEGUMES[1]

GEORGE JANSSEN[2]

University of Arkansas

Received for publication March 1, 1929

It is practically a universally accepted fact that legumes are lime-loving plants. They have come to be regarded as lime-loving, not only because they respond favorably in plant growth when grown on neutral soil but also because calcium is a necessary ion in the metabolism of the plant (13, 24). It is also known that the so-called northern legumes, particularly red clover, alfalfa, and sweet clover, are easily affected by soil acidity and that they therefore respond greatly to an application of lime when grown on acid soil. Practical agricultural experience, however, has shown that some legumes, particularly many of the so-called southern varieties, will grow in relatively acid soils without injury to the plant.

A comparative study of the various legumes which would lead to the classification of the varieties on the basis of their acid tolerance would be of value to the farmer. Possibly with this in view some investigations (3, 4, 5) have been made during the past 10 years with red clover, alsike clover, vetch, soybeans, alfalfa, and sweet clover, with the aim, among other things, of determining the exact pH values at which these plants will grow best. This work has done much to clarify thinking in respect to these legume varieties and their relation to soil acidity or alkalinity and thus has helped to give a more definite basis upon which varietal comparisons and recommendations can be made.

In view of the fact that many of the southern grown legume crops differ in variety and in growth response when grown under acid soil conditions from many of the so-called northern legumes, an investigation was undertaken to determine the comparative acid tolerance of the legumes particularly adapted to the southern states. This investigation was primarily a comparative test of the acid tolerance of some of the southern legumes.

EXPERIMENTAL METHODS

It has been shown (5, 19, 23, 24) that the plant will change the reaction of the nutrient medium in which it is growing. To maintain, therefore, a relatively constant H-ion concentration in the cultural solution, would mean

[1] Published with the approval of the dean of the college of agriculture. Research paper 136, Journal Series University of Arkansas.

[2] Assistant Agronomist, University of Arkansas.

the frequent renewal of the solutions as suggested in the above cited work, or else the introduction of an effective buffer. In order to maintain a constant H-ion concentration in the present investigation, pure Ottowa silica sand was used, to which a nutrient solution was added with or without a buffer as described later.

In the first series of the experiment 11,000 gm. of silica sand was used for each 2-gallon glazed jar. The jars were provided with a rubber stopper in which was inserted a glass tube outlet, after the method described by McCall (15). Suction applied to this outlet facilitated the removal of the old nutrient solution before the application of new. In all this work the aim was to maintain a uniform moisture content of the sand. This was done by first removing the excess of old solution by aspirating, and then bringing the jars to a definite weight by the addition of more solution.

Since it was desired to know what effect the H-ion concentration had upon the plant as well as upon the nitrogen-assimilating bacteria, two nutrient solutions were used, one containing nitrogen, the other containing no nitrogen. The plus nitrogen solution was applied to plants not inoculated whereas the minus nitrogen solution was applied to the inoculated plants. To meet the requirement in this experiment a modified form of Tarr and Noble's (23) basal solution was used. The solutions used were as follows:

	MOLECULAR CONCENTRATION	STOCK SOLUTION GRAMS IN 2000 CC.
Solution I:		
$Ca(NO_3)_2 \cdot 4H_2O$	.0182*M*	107.40
$MgSO_4 \cdot 7H_2O$	.0055*M*	33.90
KH_2PO_4	.0064*M*	21.78
K_2HPO_4	.0032*M*	13.694
Solution II:		
$CaSO_4 \cdot 2H_2O$	.0192*M*	82.0
$MgSO_4 \cdot 7H_2O$	.0055*M*	33.9
KH_2PO_4	.0064*M*	21.78
K_2HPO_4	.0032*M*	13.694

For use, 90 cc. of stock solution I, together with 3 cc. of a 0.1 per cent solution of ferric tartrate was made to a volume of 3,200 cc. with distilled water, and applied to uninoculated plants. Similarly 100 cc. of stock solution II, together with 3 cc. of a 0.1 per cent solution of ferric tartrate diluted to 3,200 cc. with distilled water, was added to the inoculated plants.

For the first series the culture solution was varied to include three H-ion concentrations as follows: pH 4.5, 5.8, and 7.0. These concentrations in every case were determined in duplicate. The pH readings were made by the colorimetric method, using the La Mott indicators. Fifth normal sulfuric acid and sodium hydroxide were used in changing the H-ion concentration of the nutrient solution. The amount of nutrient solution added every two days to

each jar ranged from 250 to 300 cc. During the first two weeks of the experiment, no buffer other than the mono- and di-potassium phosphate solution was used. This, however, did not prove sufficient to maintain the pH values during a period of 48 hours, hence a better buffer was introduced, namely, potassium acid pthalate. Tarr and Noble (23) found this a satisfactory buffer when used at a concentration of 0.005 *M*. This concentration was reduced to one-half during the latter part of the experiment. By the use of this buffer the pH values were satisfactorily maintained. No buffer was added to one of the duplicate jars which were maintained at a pH of 7.0, since it was desired to know whether it had any toxic effect on the plants. A comparison of the buffer effect can be made from these jars. It should be noted that the pH of 7.0 was relatively easily maintained, hence, no buffer was actually necessary at this reaction.

Because of the fact that the buffer was found to be toxic to some plants, another series of solution cultures was started in which no potassium acid pthalate was used but where the amount of silica sand per jar was reduced. These jars were watered daily with a very ample amount of nutrient solution. The basic material in the sand was also first removed with concentrated hydrochloric acid, and this acid in turn removed by repeated washing with water. The sand was then placed in 7-inch earthernware jars which had been coated on the inside with paraffin. A modified form of Crone's nutrient solution, the same as that used by Bryan (3), was applied to the jars. His technique was also used in making up the nutrient solution for the noninoculated plants. The modified Crone's solution contained the following chemicals:

	gm.
KCl	100
$CaCO_3 \cdot 2H_2O$	25
$MgSO_4 \cdot 7H_2O$	25
$Ca_3(PO_4)_2$	25
$FePO_4$	25

This solution formula was used for the inoculated plants, but for the uninoculated plants, the $CaCO_3 \cdot 2H_2O$ and $Ca_3(PO_4)_2$ were each reduced to 22 gm., and 25 gm. of $Ca(NO_3)_2 \cdot 7H_2O$ added. In making up either solution 12 gm. of the respective salts were weighed out, placed in a container together with 8 liters of water, thoroughly shaken, and allowed to stand 48 hours. The supernatant solution was then siphoned off and the last portion filtered. The pH was then adjusted to various values and applied daily at the rate of 100 to 150 cc. to each jar. Occasional larger quantities of nutrient solution, made to the proper H-ion concentration were added to the jars in order to help maintain a constant pH.

For the third series of this experiment silt loam field soil was used. The buffer capacity of this soil was determined according to the method used by Runk (22). The collodium bag method (20) was used for obtaining clear soil solutions. The pH of the soil was modified by the addition of 2.3 *N* HCl and

N NaOH or $Ca(OH)_2$. By determining the pH concentration at intervals of one, two, and three days as well as one and two weeks after the addition of an acid or base, it was found that the change in pH did not vary greatly after the third day. However, in this experiment, the pH readings were not taken until two weeks after the addition of acid or alkali. After this period the soil had become stabilized with respect to further pH change.

In order to obtain consistent results the soil was first passed through a 20 mesh sieve. Figure 1 gives graphically the amount of acid or base necessary to give any H-ion concentration desired in this work. In applying the acid or base, 1,300-gm. samples of soil were spread thinly on a large sheet of paper, and the acid or base was then sprayed over the soil with an atomizer. The soil was stirred intermittently. The soil was then placed in 8-inch jars and allowed

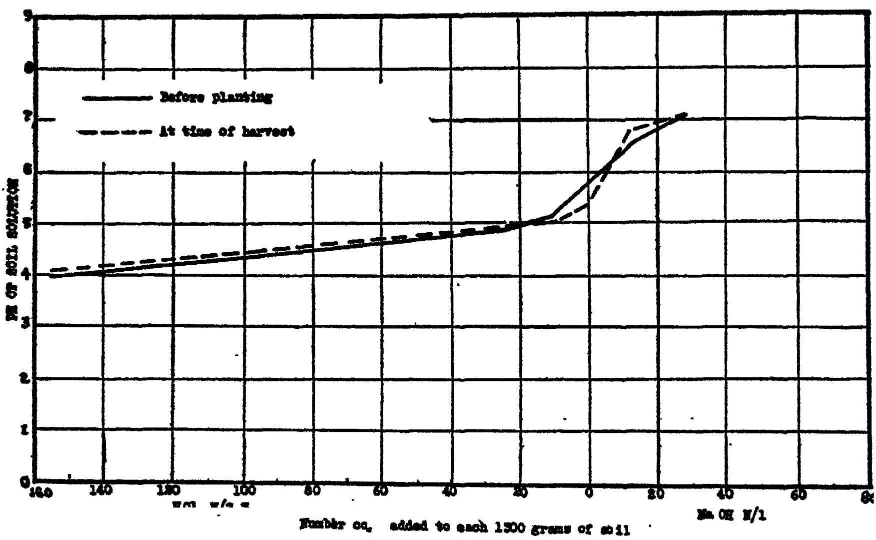


FIG. 1. CHANGE OF SOIL REACTION RESULTING FROM THE ADDITION OF AN ACID OR BASE

to stand two weeks, at which time a pH reading was taken. The legume seed was then planted and a final pH reading made at the time the experiment terminated.

For the first two series of the experiment the legume seeds were first emersed in alcohol in order to cut the surface tension so as to allow a free contact of a 1 to 1000 $HgCl_2$ solution in which they were washed. They were then thoroughly rinsed in distilled water. The seeds of the inoculated series were inoculated with the appropriate bacterial suspension and then germinated between blotters. After germination the vigorous seedlings were planted in the various jars and an additional 150 cc. of strong bacterial suspension was added to the inoculated series. The seeds planted in the plus nitrate jars received the same treatment as described, with the exception that no inocula-

tion was given. The seeds for the third series were sown very heavily in the soil without first germinating them between blotters. The plants were later thinned to six plants to each jar, the weak plants being thereby eliminated.

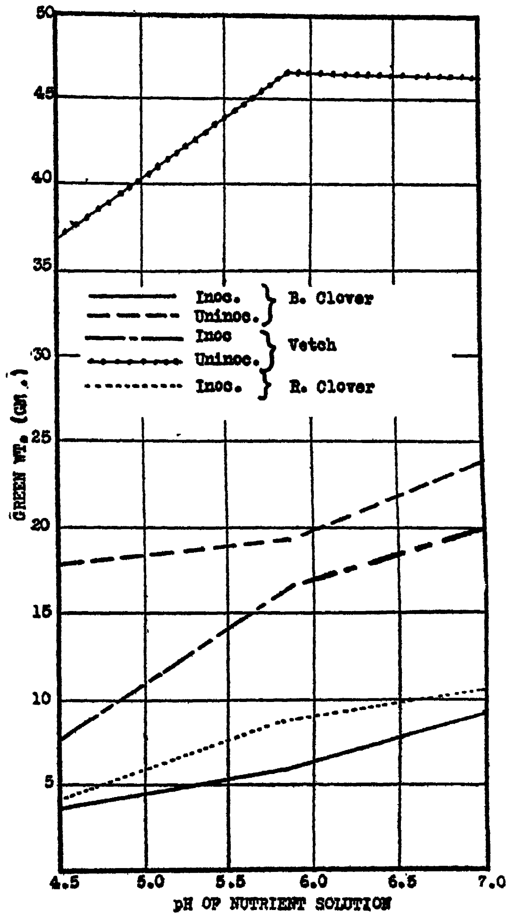


FIG. 2. GREEN WEIGHT OF LEGUME PLANTS GROWN IN SAND CULTURES WATERED WITH A MODIFIED FORM OF TARR AND NOBLE'S NUTRIENT SOLUTION MADE TO VARIOUS H-ION CONCENTRATIONS

RESULTS OF EXPERIMENT

Series I

A modified form of Tarr and Noble's (23) basal culture solution made to various H-ion concentrations was added to the jars of the first series. Red clover, California bur clover, lespedeza, and vetch were planted in these jars. The germinated seedlings were planted rather heavy and later the plants were

TABLE 1

Results from three varieties of legumes grown on nutrient solutions at pH ranges of 4.5, 5.5, and 7.0, and under two variables; inoculated without addition of nitrates; and with no inoculation but with addition of nitrates

(Modification of Tarr and Noble's Basal Solution)

JAR	pH	TREATMENT	LEGUME	TOPS, 5 PLANTS: Green weight	TOPS, 5 PLANTS: Dry weight	ROOT, DRY WEIGHT OF 5 PLANTS	RELATIVE DEGREE OF INOCULATION PER PLANT	PER CENT NITROGEN IN TOP
				gm.	gm.	gm.		
1	4.5	Inoculated. No nitrate	California bur clover	3.60	0.82	0.06	Could not be determined	2.0
3	4.5	Inoculated. No nitrate	California bur clover	3.40	0.75	0.05	Could not be determined	2.0
5	5.8	Inoculated. No nitrate	California bur clover	6.00	0.85	0.30	Could not be determined	2.4
7	5.8	Inoculated. No nitrate	California bur clover	7.00	1.09	0.30	Could not be determined	2.3
9	7.0	Inoculated. No nitrate	California bur clover	8.05	1.20	0.50	Could not be determined	3.5
11	7.0	Inoculated. No nitrate*	California bur clover	9.70	1.65	0.84	Could not be determined	2.5
2	4.5	Uninoculated. Nitrate	California bur clover	18.50	2.50	1.00	Could not be determined	Not determined
4	4.5	Uninoculated. Nitrate	California bur clover	17.00	2.55	0.95	Could not be determined	Not determined
6	5.8	Uninoculated. Nitrate	California bur clover	18.40	2.90	1.00	Could not be determined	Not determined
8	5.8	Uninoculated. Nitrate	California bur clover	20.20	3.25	1.20	Could not be determined	Not determined
10	7.0	Uninoculated. Nitrate	California bur clover	23.90	3.42	1.30	Could not be determined	Not determined
12	7.0	Uninoculated. Nitrate*	California bur clover	17.50	2.90	1.00	Could not be determined	Not determined
17	4.5	Inoculated. No nitrate	Red clover	4.32	0.70	0.20	24	2.2
15	4.5	Inoculated. No nitrate	Red clover	4.20	0.80	0.40	26	2.3
13	5.8	Inoculated. No nitrate	Red clover	5.40	0.90	0.41	40	2.5
37	5.8	Inoculated. No nitrate	Red clover	5.50	1.85	0.30	50	2.6
39	7.0	Inoculated. No nitrate*	Red clover	10.50	3.75	0.80	56	3.2
41	7.0	Inoculated. No nitrate	Red clover	3.50	0.70	0.55	50	2.8
14	4.5	Uninoculated. Nitrate	Red clover	3.70	1.15	0.16	....	Not determined
16	4.5	Uninoculated. Nitrate	Red clover	4.00	0.87	0.14	....	Not determined
18	5.8	Uninoculated. Nitrate	Red clover	5.25	1.50	0.20	....	Not determined
38	5.8	Uninoculated. Nitrate	Red clover	7.50	1.40	0.27	....	Not determined

40	7.0	Uninoculated. Nitrate	Red clover	4.50	1.00	0.20	....	Not determined
42	7.0	Uninoculated. Nitrate*	Red clover	8.00	1.60	0.45	....	Not determined
25	4.5	Inoculated. No nitrate	Hairy vetch	7.60	1.50	....	2.0	....
21	4.5	Inoculated. No nitrate	Hairy vetch	7.00	1.30	....	0.0	....
23	5.8	Inoculated. No nitrate	Hairy vetch	16.20	3.24	....	12.5	....
19	5.8	Inoculated. No nitrate	Hairy vetch	17.00	3.30	....	13.0	....
27	7.0	Inoculated. No nitrate	Hairy vetch	20.00	4.00	....	15.0	....
29	7.0	Inoculated. No nitrate*	Hairy vetch	21.00	4.20	....	24.0	....
20	4.5	Uninoculated. Nitrate	Hairy vetch	35.20	6.50	1.05	....	....
22	4.5	Uninoculated. Nitrate	Hairy vetch	40.00	8.00	0.90	....	....
24	5.8	Uninoculated. Nitrate	Hairy vetch	47.00	9.00	0.75	....	....
26	5.8	Uninoculated. Nitrate	Hairy vetch	46.00	8.90	0.55	....	....
28	7.0	Uninoculated. Nitrate	Hairy vetch	46.50	8.80	0.52	....	....
30	7.0	Uninoculated. Nitrate*	Hairy vetch	55.00	11.50	2.60	....	....

* No buffer in culture solution.

thinned to six plants to each jar. The plants were harvested 70 days after planting.

The results from this first series are given in table 1 and in figure 2. The data indicate that the greatest amount of green weight for the tops of red clover and hairy vetch grown in plus nitrate solution was obtained at a pH of 5.5 to 6.0. The same is true for the bur clover plants which were watered with an unbuffered nutrient solution; however, the buffer seems to have stimulated plant growth (see jars 10 and 12, table 1). When the above plants were grown on a minus nitrate solution but inoculated, the greatest green weights of tops were obtained at a H-ion concentration of pH 7.0. Plate 1 shows vetch plants which received a minus and a plus nitrate solution. A comparison of these plants will show that plants grown on a plus nitrate solution developed normally at a pH of 4.5 to 5.8.

The root development of both the inoculated and the uninoculated plants seems to respond better at the neutral range rather than at a higher H-ion concentration. It was difficult to determine the amount of inoculation on the roots of the plants, the percentage never being large. However, an estimate of the percentage inoculation is recorded. It is evident that the greatest inoculation was obtained when the plants were grown in a culture solution having a pH of 7.0.

The results from the lespedeza are not reported because the plants made very poor growth. However, during the brief period of plant growth it was noted that a more normal green plant color was apparent at a pH of 4.5 to 5.0 than at a pH of 7.0. It appeared from these observations that the plant actually functioned more normally in an acid medium.

Series II

As noted under methods, series II was conducted with silica sand which was washed with concentrated hydrochloric acid to remove the basic substances, and then washed with water to remove the acid. No potassium acid pthalate was added to the modified form of Crone's nutrient solution. Velvet bean, spotted bur clover, and seredella plants were used in the second series. The plants were harvested 91 days after planting.

It seems quite evident from the results of this series given in table 2 and shown graphically in figure 3, that the greatest green weight of roots and tops of the velvet bean was made at a pH of 5.8 to 6. This was true for both the inoculated and the uninoculated plants. The reader should be reminded that the cotyledons of the velvet bean are very large and it may be that this latent plant-food stored in the seed affects the plant's capacity to grow in an acid medium.

The seredella and bur clover plants (fig. 3), like the velvet bean, made a greater total green weight of tops and roots at a pH of 5.6 than at 7.0. The cotyledons on the seeds are very small and should therefore not be a serious factor in altering the tolerance of the plant when grown on an acid medium.

Plate 2 shows the relationship of plant growth of seredella and bur clover as affected by hydrogen-ion concentration.

Series III

In series III the plants were grown on a silt loam soil, the pH of which was changed as discussed under methods. In this experiment the soil was adjusted to include seven different pH values. To vary the hydrogen-ion concentration

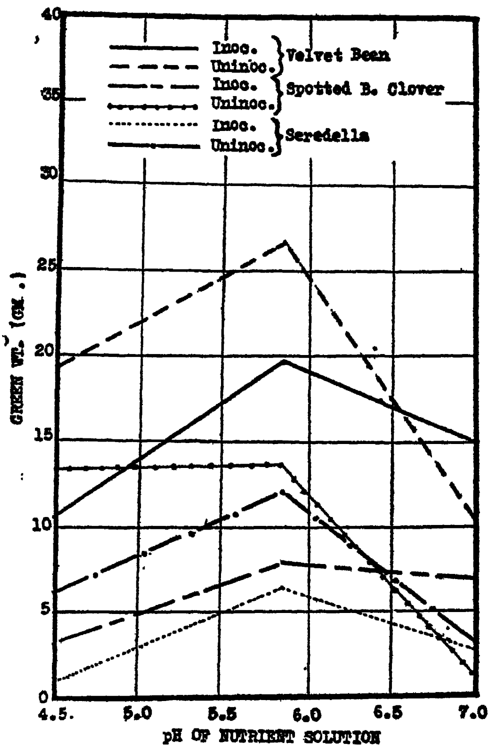


FIG. 3. GREEN WEIGHT OF LEGUME PLANTS GROWN IN SAND CULTURES WATERED WITH CRONE'S NUTRIENT SOLUTION MODIFIED TO INCLUDE VARYING H-ION CONCENTRATION

2.3 *N* hydrochloric acid was used, whereas *N* sodium hydroxide or calcium hydroxide was used for varying the OH ion. Calcium hydroxide was used in comparison with the sodium hydroxide at a pH of 7.0 only.

It appeared to the writer that when plants are grown in culture solutions, the pH values of which are modified so as to include various H-ion concentrations, the natural root environment of the plant is completely changed, and that the results obtained from a comparison of acid tolerance of legumes under such conditions, would not be similar to that which would be obtained in

TABLE 2

Results on the growth of three southern legumes grown at pH values of 4.6, 5.8, and 7.0 on silica sand and watered with Crone's nutrient solution

JAR	pH	TREATMENT	LEGUME	TOPS, 5 PLANTS: Green weight	TOPS, 5 PLANTS: Dry weight	ROOT, DRY WEIGHT OF 5 PLANTS	RELATIVE DEGREE OF INOCULATION ON 100 PER CENT
				gm.	gm.	gm.	
49	4.5	Inoculated. No nitrate	Velvet beans*	9.2	1.5	1.70	0
50	4.5	Inoculated. No nitrate	Velvet beans	11.7	2.0	3.00	0
53	5.8	Inoculated. No nitrate	Velvet beans	19.7	4.0	2.50	5
54	5.8	Inoculated. No nitrate	Velvet beans	20.1	4.5	1.90	10
57	7.0	Inoculated. No nitrate	Velvet beans	16.0	3.4	3.00	40
58	7.0	Inoculated. No nitrate	Velvet beans	14.0	3.0	1.70	50
51	4.5	Uninoculated. Nitrate	Velvet beans	19.0	4.0	2.17	Not inoculated
52	4.5	Uninoculated. Nitrate	Velvet beans	20.0	4.2	2.17	Not incoulated
55	5.8	Uninoculated. Nitrate	Velvet beans	28.5	6.0	2.95	Not inoculated
56	5.8	Uninoculated. Nitrate	Velvet beans	25.3	5.3	2.95	Not inoculated
59	7.0	Uninoculated. Nitrate	Velvet beans	12.5	1.5	1.05	Not inoculated
60	7.0	Uninoculated. Nitrate	Velvet beans	9.0	2.0	1.05	Not inoculated
61	4.5	Inoculated. No nitrate	Bur clover (spotted)	3.0	1.4	0.20	40
62	4.5	Inoculated. No nitrate	Bur clover (spotted)	3.1	1.8	0.20	50
65	5.8	Inoculated. No nitrate	Bur clover (spotted)	7.5	1.9	0.40	50
66	5.8	Inoculated. No nitrate	Bur clover (spotted)	8.7	2.0	0.50	80
69	7.0	Inoculated. No nitrate	Bur clover (spotted)	5.4	1.7	0.50	90
70	7.0	Inoculated. No nitrate	Bur clover (spotted)	8.7	2.0	0.50	100
63	4.5	Uninoculated. Nitrate	Bur clover (spotted)	15.9	3.0	1.95	Not inoculated
64	4.5	Uninoculated. Nitrate	Bur clover (spotted)	11.0	2.9	1.95	Not inoculated
67	5.8	Uninoculated. Nitrate	Bur clover (spotted)	13.5	3.2	0.90	Not inoculated
68	5.8	Uninoculated. Nitrate	Bur clover (spotted)	13.6	3.0	0.90	Not inoculated
70	7.0	Uninoculated. Nitrate	Bur clover (spotted)	1.5	0.6	0.10	Not inoculated
71	7.0	Uninoculated. Nitrate	Bur clover (spotted)	1.3	0.5	0.10	Not inoculated
73	4.5	Inoculated. No nitrate	Seredella	1.8	0.2	0.75	5

74	4.5	Inoculated. No nitrate	Seredella	1.2	0.2	0.75	5
77	5.8	Inoculated No nitrate	Seredella	6.0	1.8	1.40	20
78	5.8	Inoculated. No nitrate	Seredella	7.0	1.9	1.25	20
81	7.0	Inoculated. No nitrate	Seredella	4.2	0.7	1.40	40
82	7.0	Inoculated. No nitrate	Seredella	3.9	0.9	1.00	40
75	4.5	Uninoculated. Nitrate	Seredella	7.0	1.8	1.15	Not inoculated
76	4.5	Uninoculated. Nitrate	Seredella	6.0	1.5	1.15	Not inoculated
79	5.8	Uninoculated. Nitrate	Seredella	9.0	2.5	1.75	Not inoculated
80	5.8	Uninoculated. Nitrate	Seredella	15.0	3.2	1.75	Not inoculated
83	7.0	Uninoculated. Nitrate	Seredella	4.6	0.7	1.45	Not inoculated
84	7.0	Uninoculated. Nitrate	Seredella	3.9	0.4	1.45	Not inoculated

* Three plants.

'ABL'

Comparative acid tolerance of some southern varieties of legumes grown on soil having varying degrees of acidity

JAR NUMBER	VARIETY	TREATMENT	pH BEGINNING	pH END	WEIGHT PER 5 PLANTS, TOPS	WEIGHT PER 5 PLANTS, ROOTS	HEIGHT	TYPE OF GROWTH	EMERGENCE*	RELATIVE DEGREE OF INOCULATION
					gm.	*gm.*	*inches*			
3	Bur clover (spotted)	$Ca(OH)_2$	7.0	6.5	1.60	1.65	4	Good	January 20, 1928	Excellent
4	Bur clover (spotted)	$Ca(OH)_2$	7.0	6.5	1.35	2.25	4	Good	January 20, 1928	Excellent
15	Bur clover (spotted)	NaOH	7.0	6.5	1.50	1.20	3	Good	January 20, 1928	Excellent
16	Bur clover (spotted)	NaOH	7.0	6.6	1.50	1.20	3.5	Good	January 20, 1928	Excellent
19	Bur clover (spotted)	NaOH	6.5	6.0	1.00	1.00	3	Fair	January 20, 1928	Fair
20	Bur clover (spotted)	NaOH	6.5	6.0	1.00	1.75	3	Fair	January 20, 1928	Fair
45	Bur clover (spotted)	0	5.9	5.4	0.20	0.30	1	Poor	January 18, 1928	0
46	Bur clover (spotted)	0	5.9	5.4	0.25	0.40	1	Poor	January 18, 1928	0
23	Bur clover (spotted)	HCl	4.9	4.7	0.31	0.31	2	Poor	January 19, 1928	0
24	Bur clover (spotted)	HCl	4.9	4.8	0.30	0.40	2	Poor	January 19, 2928	0
7	Bur clover (spotted)	HCl	4.6	4.4	0.30	0.45	2	Poor	January 19, 2928	0
8	Bur clover (spotted)	HCl	4.6	4.6	0.50	0.46	2	Poor	January 19, 1928	0
11	Bur clover (spotted)	HCl	4.0	4.2	0.20	0.30	2	Poor	January 19, 1928	0
12	Bur clover (spotted)	HCl	4.0	4.2	0.20	0.25	2	Poor	January 19, 1928	0
41	Crimson clover	NaOH	7.0	6.5	2.10	1.00	4	Excellent	January 7, 1928	Good
42	Crimson clover	NaOH	7.0	6.5	2.00	2.30	5	Excellent	January 7, 1928	Good
37	Crimson clover	NaOH	6.5	6.2	3.00	1.70	5	Excellent	January 7, 1928	Good
38	Crimson clover	NaOH	6.5	6.2	3.10	1.50	5	Excellent	January 7, 1928	Good
49	Crimson clover	0	5.9	5.4	3.30	2.50	5	Excellent	January 5, 1928	Excellent
50	Crimson clover	0	5.9	5.4	2.00	2.50	3	Excellent	January 5, 1928	Excellent
33	Crimson clover	HCl	4.6	4.7	0	0	0	Poor	January 6, 1928	0
34	Crimson clover	HCl	4.6	4.7	0	0	0	Poor	January 6, 1928	0
29	Crimson clover	HCl	4.0	4.4	0	0	0	Poor	January 6, 1928	0
30	Crimson clover	HCl	4.0	4.3	0	0	0	Poor	January 6, 1928	0
39	Seredella	NaOH	7.0	6.5	0	0	0	0	0	0

40	Seredella	NaOH	7.0	6.5	0	0	0	0	0	0
35	Seredella	NaOH	6.5	6.0	0.20	0.20	1	Poor	January 8, 1928	0
36	Seredella	NaOH	6.5	6.0	0.15	0.15	1	Poor	January 8, 1928	0
47	Seredella	0	5.9	5.4	0.50	0.50	3	Fair	January 6, 1928	Excellent
48	Seredella	0	5.9	5.4	0.40	0.35	3	Fair	January 6, 1928	Excellent
31	Seredella	HCl	4.6	4.6	0.17	0.10	2	Fair	January 6, 1928	Few
32	Seredella	HCl	4.6	4.6	0.23	0.20	2	Fair	January 6, 1928	Few
27	Seredella	HCl	4.0	4.2	0.12	0.10	2	Fair	January 7, 1928	None
28	Seredella	HCl	4.0	4.2	0.085	0.05	2	Fair	January 7, 1928	None
76	Hairy vetch	$Ca(OH)_2$	7.0	7.4	2.20	2.00	8	Fair	February 16, 1928	Good
77	Hairy vetch	$Ca(OH)_2$	7.0	7.4	2.20	2.30	8	Fair	February 16, 1928*	Good
52	Hairy vetch	NaOH	7.0	7.3	2.60	1.60	8	Fair	February 16, 1928	Good
53	Hairy vetch	NaOH	7.0	7.0	3.20	1.90	12	Fair	February 16, 1928	Good
64	Hairy vetch	NaOH	6.5	6.2	6.00	3.00	13	Good	February 16, 1928	Excellent
65	Hairy vetch	NaOH	6.5	6.2	7.5	2.5	18	Good	February 16, 1928	Excellent
88	Hairy vetch	0	5.9	5.4	4.0	2.9	14	Fair	February 16, 1928	Excellent
89	Hairy vetch	0	5.9	5.4	4.3	3.5	18	Fair	February 16, 1928	Good
100	Hairy vetch	HCl	5.3	5.2	3.8	2.5	11	Fair	February 16, 1928	Good
101	Hairy vetch	HCl	5.3	5.2	3.7	2.5	13	Fair	February 16, 1928	Good
112	Hairy vetch	HCl	4.9	5.0	3.2	2.4	11	Fair	February 16, 1928	Good
113	Hairy vetch	HCl	4.9	5.0	3.2	2.0	12	Fair	February 16, 1928	Good
124	Hairy vetch	HCl	4.6	4.7	4.0	1.7	10	Fair	February 20, 1928	Good
125	Hairy vetch	HCl	4.6	4.7	4.0	2.0	12	Fair	February 20, 1928	Few
136	Hairy vetch	HCl	4.0	4.2	1.6	1.6	4	Poor	February 24, 1928	0
137	Hairy vetch	HCl	4.0	4.2	1.4	2.0	4	Poor	February 24, 1928	0
78	Canadian field peas	$Ca(OH)_2$	7.0	7.4	1.5	0.5	19	Poor	February 18, 1928	Poor
79	Canadian field peas	$Ca(OH)_2$	7.0	7.4	1.7	1.5	20	Poor	February 18, 1928	Fair
54	Canadian field peas	NaOH	7.0	7.0	4.05	1.2	36	Good	February 18, 1928	Fair
55	Canadian field peas	NaOH	7.0	7.0	3.3	1.05	34	Good	February 18, 1928	Fair

* Bur clover, crimson clover, and seredella planted January 14, 1928, harvested April 1.
Vetch, sweet clover, Canadian peas, Austrian peas planted February 12, 1928, harvested May 2.

TABLE 3—*Continued.*

JAR NUMBER	VARIETY	TREATMENT	pH BEGINNING	pH END	WEIGHT PER 5 PLANTS, TOPS	WEIGHT PER 5 PLANTS, ROOTS	HEIGHT	TYPE OF GROWTH	EMERGENCE*	RELATIVE DEGREE OF INOCULATION
					gm.	gm.	inches			
66	Canadian field peas	NaOH	6.5	6.5	7.05	2.1	46	Excellent	February 18, 1928	Excellent
67	Canadian field peas	NaOH	6.5	6.5	5.0	1.9	36	Excellent	February 18, 1928	Excellent
90	Canadian field peas	0	5.9	5.4	5.2	2.0	40	Good	February 18, 1928	Fair
91	Canadian field peas	0	5.9	5.4	4.4	1.9	32	Good	February 18, 1928	Fair
102	Canadian field peas	HCl	5.3	5.3	3.3	1.2	23	Good	February 16, 1928	Fair
103	Canadian field peas	HCl	5.3	5.3	3.6	1.5	31	Good	February 16, 1928	Fair
114	Canadian field peas	HCl	4.9	4.9	1.8	1.5	22	Fair	February 18, 1928	Medium
115	Canadian field peas	HCl	4.9	5.0	1.81	1.2	17	Fair	February 18, 1928	Medium
126	Canadian field peas	HCl	4.6	4.8	1.5	0.9	16		February 18, 1928	Poor
127	Canadian field peas	HCl	4.6	4.8	1.4	0.8	15		February 18, 1928	Poor
138	Canadian field peas	HCl	4.0	4.3	0	0	0	0	February 21, 1928	0
139	Canadian field peas	HCl	4.0	4.2	0	0	0	0	February 21, 1928	0
80	Austrian field peas	$Ca(OH)_2$	7.0	7.5	3.1	3.1	10	Fair	February 16, 1928	Few
81	Austrian field peas	$Ca(OH)_2$	7.0	7.4	2.9	2.9	10	Fair	February 16, 1928	Few
56	Austrian field peas	NaOH	7.0	7.0	2.8	1.9	9	Fair	February 20, 1928	
57	Austrian field peas	NaOH	7.0	7.0	2.5	1.7	7	Fair	February 20, 1928	Good
68	Austrian field peas	NaOH	6.5	6.8	5.6	2.4	16	Good	February 16, 1928	Excellent
69	Austrian field peas	NaOH	6.5	6.6	5.7	2.0	23	Good	February 16, 1928	Excellent
92	Austrian field peas	0	5.9	5.4	3.0	1.6	12	Good	February 16, 1928	Good
93	Austrian field peas	0	5.9	5.4	4.9	2.7	15	Good	February 16, 1928	Good
104	Austrian field peas	HCl	5.3	5.2	3.5	2.4	16	Good	February 14, 1928	Fair
105	Austrian field peas	HCl	5.3	5.2	2.8	1.9	13	Good	February 14, 1928	Fair
116	Austrian field peas	HCl	4.9	4.8	2.5	1.4	7	Good	February 16, 1928	Fair
117	Austrian field peas	HCl	4.9	4.9	2.4	1.4	14	Good	February 16, 1928	Fair
128	Austrian field peas	HCl	4.6	4.6	1.5	1.0	9		February 18, 1928	None
129	Austrian field peas	HCl	4.6	4.7	0.96	1.0	5		February 18, 1928	None

140	Austrian field peas	HCl	4.0	4.4	0.81	1.6	3		February 21, 1928	None
141	Austrian field peas	HCl	4.0	4.2	0.4	1.5	2		February 21, 1928	None
82	Subterranean clover	$Ca(OH)_2$	7.0	7.6	3.0	3.0	2	Fair	February 14, 1928	Poor
83	Subterranean clover	$Ca(OH)_2$	7.0	7.5	3.0	3.5	2	Fair	February 14, 1928	Poor
58	Subterranean clover	NaOH	7.0	7.0	3.8	2.2	2.5	Fair	February 14, 1928	Good
59	Subterranean clover	NaOH	7.0	7.0	5.0	2.6	3.5	Fair	February 14, 1928	Good
70	Subterranean clover	NaOH	6.5	6.5	5.1	2.7	4	Excellent	February 14, 1928	Good
71	Subterranean clover	NaOH	6.5	6.4	5.5	3.0	3	Excellent	February 14, 1928	Fair
94	Subterranean clover	0	5.9	5.5	5.0	2.9	3.5	Excellent	February 14, 1928	Good
95	Subterranean clover	0	5.9	5.6	4.5	3.2	2.5	Excellent	February 14, 1928	Medium
106	Subterranean clover	HCl	5.3	5.2	5.5	4.9	3.0	Excellent	February 14, 1928	Medium
107	Subterranean clover	HCl	5.3	5.4	4.7	4.6	2.5	Excellent	February 14, 1928	Medium
118	Subterranean clover	HCl	4.9	5.2	7.2	6.7	3.5	Excellent	February 14, 1928	Medium
119	Subterranean clover	HCl	4.9	5.2	5.0	7.0	2.5	Excellent	February 14, 1928	Medium
130	Subterranean clover	HCl	4.6	4.7	2.8	2.5	2.5	Fair	February 14, 1928	Medium
131	Subterranean clover	HCl	4.6	4.8	3.8	3.4	3	Fair	February 14, 1928	Medium
142	Subterranean clover	HCl	4.0	4.4	1.0	0.8	1.5	Poor	February 16, 1928	Poor
143	Subterranean clover	HCl	4.0	4.2	1.2	1.3	1.5	Poor	February 16, 2928	Poor
84	White sweet clover biennial	$Ca(OH)_2$	7.0	7.6	2.5	2.7	3.5	Fair	February 14, 1928	Fair
85	White sweet clover biennial	$Ca(OH)_2$	7.0	7.5	2.5	2.5	3.0	Fair	February 14, 1928	Fair
60	White sweet clover biennial	NaOH	7.0	6.8	3.2	3.5	8	Excellent	February 16, 1928	Good
61	White sweet clover biennial	NaOH	7.0	6.8	3.0	3.0	4	Excellent	February 16, 1928	Good
72	White sweet clover biennial	NaOH	6.5	6.7	3.2	3.2	8	Excellent	February 14, 1928	Medium
73	White sweet clover biennial	NaOH	6.5	6.6	3.6	4.5	6	Excellent	February 14, 1928	Medium
96	White sweet clover biennial	0	5.9	5.6	2.4	2.0	4	Good	February 14, 1928	Medium
97	White sweet clover biennial	0	5.9	5.6	2.8	2.0	6	Good	February 14, 1928	Medium
108	White sweet clover biennial	HCl	5.3	5.4	2.2	2.5	5	Fair	February 18, 1928	Medium
109	White sweet clover biennial	HCl	5.3	5.4	2.2	2.7	4.5	Fair	February 18, 1928	Medium
120	White sweet clover biennial	HCl	4.9	5.2	2.0	2.9	4.0	Poor	February 18, 1928	Medium

TABLE 3—*Continued*

JAR UMBER	VARIETY	TREATMENT	pH BEGINNING	pH END	WEIGHT PER 5 PLANTS, TOPS	WEIGHT PER 5 PLANTS, ROOTS	HEIGHT	TYPE OF GROWTH	EMERGENCE*	RELATIVE DEGREE OF INOCULATION
					gm.	*gm.*	*inches*			
121	White sweet clover biennial	HCl	4.9	5.2	1.7	2.1	3.5	Poor	February 18, 1928	Medium
132	White sweet clover biennial	HCl	4.6	4.7	0.6	0.7	1.5	Poor	February 20, 1928	Poor
133	White sweet clover biennial	HCl	4.6	4.6	1.1	0.9	1.5	Poor	February 20, 1928	Poor
144	White sweet clover biennial	HCl	4.0	4.2	0	0	0		February 21, 1928	0
145	White sweet clover biennial	HCl	4.0	4.2	0	0	0		February 21, 1928	0
86	Hubam	$Ca(OH)_2$	7.0	7.7	3.1	2.7	9	Fair	February 15, 1928	Fair
87	Hubam	$Ca(OH)_2$	7.0	7.8	1.8	2.0	6	Fair	February 15, 1928	Fair
62	Hubam	NaOH	7.0	7.1	4.0	2.6	16	Good	February 18, 1928	Good
63	Hubam	NaOH	7.0	7.2	4.5	3.0	15	Good	February 18, 1928	Good
74	Hubam	NaOH	6.5	6.6	4.2	2.0	15	Good	February 15, 1928	Good
75	Hubam	NaOH	6.5	6.6	4.7	2.0	17	Good	February 15, 1928	Good
98	Hubam	0	5.9	5.6	4.0	2.9	12	Good	February 15, 1928	Good
99	Hubam	0	5.9	5.6	4.3	3.5	14	Good	February 15, 1928	Good
110	Hubam	HCl	5.3	5.4	4.0	2.2	15	Fair	February 19, 1928	Fair
111	Hubam	HCl	5.3	5.4	4.2	2.5	11	Fair	February 19, 1928	Fair
122	Hubam	HCl	4.9	5.2	1.7	1.7	4	Poor	February 19, 1928	0
123	Hubam	HCl	4.9	5.0	2.7	2.4	9	Poor	February 19, 1928	0
134	Hubam	HCl	4.6	4.6	0.8	0.5	2	Poor	February 19, 1928	0
135	Hubam	HCl	4.6	4.5	0.6	0.5	4	Poor	February 19, 1928	0
146	Hubam	HCl	4.0	4.2	0.0	0.0	0	0	February 19, 1928	0
147	Hubam	HCl	4.0	4.2	0.0	0.0	0	0	February 19, 1928	0
	Soybean†	NaOH	8.0	7.0	2.0		8	Fair	November 5, 1927	0
	Soybean	NaOH	7.0	6.4	4.0		10	Good	November 5, 1927	Few
	Soybean	0	5.9	5.6	5.0		12	Good	November 4, 1927	Excellent
	Soybean	HCl	4.5	5.0	1.5		6	Fair	November 4, 1927	0
	Soybean	HCl	3	4.2	0.5		1	Poor	November 4, 1927	0

† Planted November 1, 1927, harvested December 20, weight of 3 plants.

ordinary field soil studies. For this reason the present experiment was conducted in order to make a comparison with the previous sand cultures.

The following legumes were used in this series of experiments. Spotted bur clover, lespedeza, crimson clover, seredella, hairy vetch, Canadian peas, Austrian peas, subterranean clover, Hubam clover, and biennial white sweet clover. The bur clover, crimson clover, and seredella were harvested 77 days after planting whereas the remainder of the varieties were grown 80 days before they were harvested.

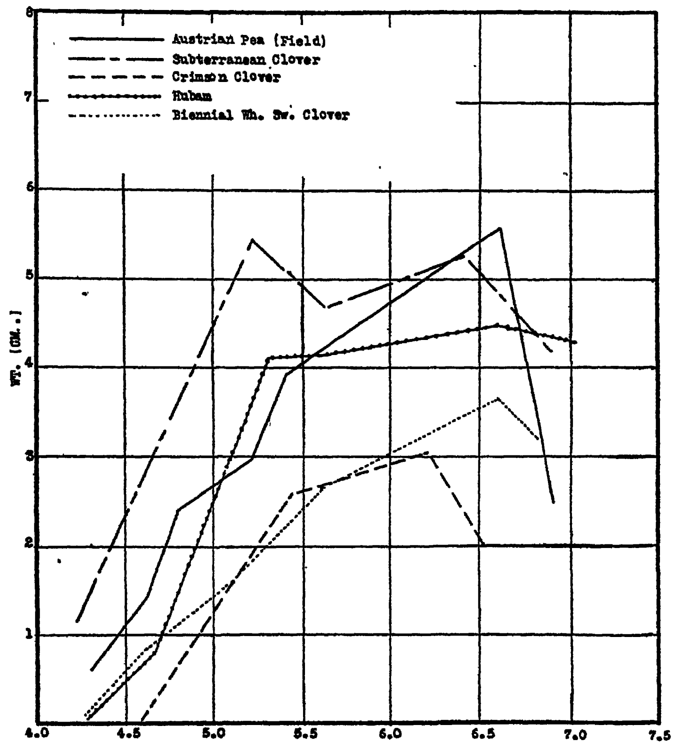


FIG. 4. DRY WEIGHT OF LEGUME PLANTS GROWN ON CLARKSVILLE SILT LOAN, THE pH OF WHICH WAS ARTIFICIALLY MODIFIED

The results from this series are given in table 3. Relative to the rate of plant emergence it will be noted that in most cases a pH of 5.5 to 6.0 was most favorable to the plant. The extreme acid range as well as a slightly alkaline range delayed plant emergence in most cases. The fact that when seeds were sown in an alkaline sand culture they germinated and emerged more slowly than seeds planted at a neutral range, was also noted by Theron (24). Apparently, however, the relationship between the rate of plant emergence and the acidity or alkalinity of the cultural solution in which the seeds are planted depends

somewhat on the variety of seeds used, since, as noted in the table, the Austrian field peas emerged more quickly at a pH of 5.3 than at 7.0, whereas the Hubam clover emerged as soon at a pH of 7.0 as at other ranges on the pH scale.

The dry weights of tops of the various legumes are shown graphically in figures 4 and 5. It is evident from these dry weights that the optimum pH for most of the legumes worked with in soil was 6 to 6.5. Certain legumes, however, as the seredella, spotted bur clover, and possibly the subterranean

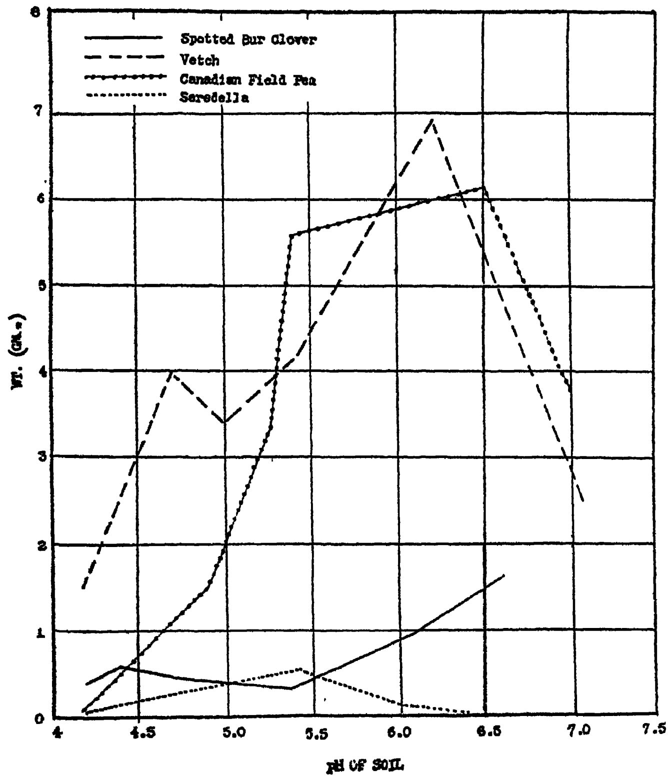


FIG. 5. DRY WEIGHT OF LEGUME PLANTS GROWN ON CLARKSVILLE SILT LOAM, THE pH OF WHICH WAS ARTIFICIALLY MODIFIED

clover grew as well at a decidedly higher H-ion concentration than the legumes previously named. It appears also that varietal differences with reference to acid tolerance exist within the same species of plants. Thus although sweet clover grows best at a pH of about 6.5 to 7.0 the Hubam sweet clover seems to have a wider range of acid tolerance and may make a fair growth at a pH of 5.

Plate 3 shows clearly the difference in growth response with respect to the H-ion concentration of various legumes. It will be noted that the bur clover and Austrian field pea made very good growth at a pH of 4.5, whereas the sweet clover required a soil with a pH of 7.0 for its best growth.

DISCUSSION

Coville (6) stated that the cowpea, hairy vetch, crimson clover, soybean, lupine, and seredella are useful on acid soil types. Popular agricultural opinion also conveys the idea that the above named legumes are to a considerable degree acid tolerant and therefore particularly adapted to many of the acid soil regions of the south. The question arizes, is this assumption correct? If so, what is the relationship of these varieties on the basis of a comparative acidity test? Furthermore has the soil reaction an effect on the plant or on the nitrogen assimilating bacteria or both? A good many investigators (5, 6, 11, 12, 21, 22, 23, 24) have shown that there are differences in the capacity of the plants to grow on acid soils. Other workers (8, 9) have found that there are differences in the nitrogen assimilating bacteria with respect to their ability to grow in an acid or alkaline medium. MacIntire (16) believes that the harmful effect of soil acidity is not on the plant but that it may effect the nitrogen assimilating bacteria. Truog (25) has shown that the harmful effect of an acid soil is due to the lack of calcium available to the plant. Bryan (3) further found that the greater the acidity of the nutrient solution the less power the plant has of obtaining its calcium for growth processes. Other workers (10, 17, 18) have shown that substances in solution in an acid soil may be toxic to the plant. The problem, therefore, of soil acidity and the reason for its injurious effects on the plant is a complex one in which many factors operate. The present work is not an attempt to deal with the harmful effects of an acid medium on the plant but a comparative study of some southern legumes with relation to their tolerance to soil acidity.

It was noted previously that the mono- and di-potassium phosphates in the nutrient solution of series I were not sufficient buffer to maintain a constant H-ion concentration. Even when 300 cc. of nutrient solution was added to the jars daily the pH of the solution was not maintained. This was no doubt because of the large amount of sand used, together with the fact that the basic substances had not been removed. When this basic material was removed with concentrated HCl as was done for sand used in the second series, it was found that the H ion concentration could be maintained if the culture solutions were applied daily.

By adding potassium acid phthalate, in concentration of .0052 M, the pH values were fairly well maintained. By comparing the green weight of plants from jars which received nitrate and were kept at a pH of 7.0, but which received the buffer solution, with the jar not receiving the buffer, it will be noted that the potassium acid phthalate reduced the green weight of red clover 40.3 per cent and that of vetch 15.6 per cent. On the other hand the green weight of the California bur clover (not inoculated) was increased when the buffer solution was added, whereas the inoculated plants were little affected. These results on the toxicity of the phthalate buffer agree with the findings of Dustiman (7) who found toxicity to the tomato and barley plants when 500 ppm. of potassium acid pthalate was added to the nutrient solution.

The toxicity of the buffer solution to red clover plants was greater when they were grown at a pH of 7.0 than at a higher H ion concentration. This might partially be due to an unbalanced condition of the nutrient solution at a pH of 7.0. It has been shown by Tarr and Noble (23) that about eight-ninths of the calcium and less than one-half of the magnesium precipitated from solutions between the pH values of 3 and 7.59. It appears that in this series, particularly for plants receiving nitrates, the precipitation of much of the calcium in excess of magnesium from the solution at a pH of 7 tended, together with the buffer solution, to bring about greater toxicity than was obtained at a pH of 4.5 and 5.8 when the Ca and Mg concentration were present in a more balanced relation. Such a balanced relation was noted by Loew (14) to be of extreme importance to the permeability of the plant. It is very evident in work pertaining to the H-ion concentration in plant growth that analyses be made (23) of the salts remaining in solution at the various pH values in order to maintain a uniform salt concentration through the series. In the second series of these experiments the precipitation of salts from solution was noted in the lack of sufficient iron in the medium at the neutral point, and consequently the plants became chlorotic. However, the addition of 5 cc. of a 0.1 per cent solution of ferric tartrate tended to bring the plants back to normal color even at a pH of 7.

In making a comparison of the uninoculated red clover and vetch plants, it will be seen that the greatest growth on a green weight basis was obtained at a pH of 5.8. On the other hand the inoculated plants made their greatest growth at a pH of 7.0. This would offer one of two suggestions: first, that the nitrogen assimilating bacteria were retarded in their development at the lower pH value; or second, that the plants were not sufficiently inoculated in their early development. If we note, however, that a greater percentage of inoculation occurred at a pH of 7 than at a lower pH range, it becomes apparent that the first suggestion is in order, namely, that the bacterial growth was retarded at the lower pH value and concomitantly plant growth was also retarded. It will also be seen that the plants receiving nitrates made as good growth at a pH of 5.6 as at 7.0. These results on the growth of the nitrogen-assimilating bacteria are in agreement with Fred's (8) findings on the growth of the alfalfa bacteria in pure culture at varying pH values. The difference in growth response of the velvet bean and vetch plant to varying H-ion concentration may be observed in plates 1 and 2.

The results obtained from a comparative test of legumes grown in sand culture solutions here reported indicate the following gradient of low to high acid tolerance: red clover, vetch, seredella, California bur clover, spotted bur clover, and velvet bean.

The data from the third series of the experiment in which the soil reaction was changed by the addition of a 2.3 *N* acid or *N* base are comparative only, since the reaction in the soil could not be kept constant throughout the entire period of plant growth. The reader must also keep in mind that no conclusions

can be given with respect to modifying factors on plant growth, such as aluminum, magnesium, or iron toxicity in the acid range, because these substances are brought into solution. All that can be said is that the pH was determined at the time the seed was planted and again at harvest. Nevertheless, as previously intimated, the results are comparative and, in the mind of the writer, the plant growth was more normal and conclusive than that which could be obtained in sand or water cultures.

The data on the germination of the various legumes, particularly the sweet clover, show that the seed will germinate in very acid soil but that the plant dies soon afterwards. Some of the larger type of seed, such as the Canadian field pea, also germinate readily and may even produce good growth in an acid soil, possibly because of the readily available food in the cotyledons, but sooner or later the plants turn yellow and die. These results are somewhat contrary to those of Joffe (12), who found that alfalfa plants, even at a pH of 3.5, when once established had a normal green color and made good growth. The soil used in the present experiment was very low in total nitrogen. However, had there been a greater supply of available nitrogen, these plants also might have developed at a higher H-ion concentration.

It appears that the plants here tested grew better at a pH of 6 to 6.5 than at a pH of 7.0. The total dry weight of the plants as shown by figure 3 and plate 2 was much reduced at a pH of 7.0. It appears that when $Ca(OH)_2$ was used to change the soil reaction to neutrality the plants did not grow as well as when NaOH was used for this purpose (see table 3 and figures 4 and 5) in spite of the fact that a much better physical condition of the soil obtained with $Ca(OH)_2$. This better growth when NaOH was used to change the soil reaction was particularly noted in the case of the sweet clovers. This seems to be in agreement with the fact that these plants habitually grow well in alkali regions. Results from field tests at this station have also shown that legume plants grown on soils similar to that used by the writer in this experiment do not respond to lime. These results suggest that the soil when made neutral with $Ca(OH)_2$ had such a high lime content that it tended to precipitate the phosphate and that this becomes the limiting factor in plant growth, and therefore only legumes with a high lime requirement (26) would be expected to respond to this treatment.

Breaux (2) noted that when lime was added to acid soils, the neutrality was not long maintained but that a change occurred in pH within 24 hours. He noted that after a period of three months the pH had changed over a range of 0.5 to 1.5 units below the initial value produced by liming. In the present experiment the pH from the beginning to the time the experiment terminated varied from 0 to 0.5, depending on the H-ion concentration of the soil at the time the experiment was started. When the pH of the soil was artificially altered from the normal of 5.9 to that of 7.0 or 7.5 then during the period of the experiment a change of 0.5 pH was noted, but when the normal pH of the soil was not changed over 0.5 to 1.0 very little change in pH occurred during the

period of the experiment, though fluctuations may have occurred during the term of the experiment. Bauer (1) noted variations in the H-ion concentration of soils with different moisture contents and he suggested that these periodic pH fluctuations should be considered in soil acidity studies. Such periodic measurements were not made in this experiment, but the extent of such changes from time to time would depend upon the buffer capacity of the soil.

A study of figures 4 and 5 will show that there is a marked variation in the capacity of the various legumes to grow on acid soils. Seredella and subterranean clover were particularly able to make good growth in a relatively acid medium whereas the sweet clovers grew better in a more neutral soil. The range of acid tolerance for this last series in which the pH of the soil was modified from high to low acidity is as follows: seredella, subterranean clover, vetch, bur clover, Austrian field pea, soybean, Canadian field pea, crimson clover, Hubam clover, and biennial white sweet clover.

In experiments concerned with symbiotic forms, such as are reported here, one questions how the host can adapt itself to a relatively acid soil when the bacterial organism prefers a neutral medium. To this effect Fred (9) has shown that some forms of the nitrogen assimilating bacteria, as the lupine form, are able to make good growth at as low a pH as 3.2 whereas such forms as alfalfa bacteria can develop at a pH no higher than 4.9. Thus it may be partially for this reason that the seredella plant, for instance, can make better growth in acid soil than some of the other forms of legumes here used. However, it appears that if the soil is well supplied with nitrogen, many of the legumes here tested can make considerable growth in a comparatively acid soil.

SUMMARY

The present investigation is a comparative acid tolerance test of southern legumes. The legumes were grown in a modified form of Tarr and Noble's basal culture solution with and without potassium acid phthalate; modified form of Crone's solution; and Clarksville silt loam soil, the pH of which was changed by addition of an acid base.

1. It was found that, when 10 kgm. of pure silica sand was used, it was impossible to maintain a constant pH, even if the culture solution was added daily in large amounts. Potassium acid pthalate used at 0.0052*M* strength during the first half of the experiment and one-half that amount during the last half of the test, tended to keep the pH constant if the nutrient solutions were added daily or every 48 hours.

2. Toxicity of potassium acid pthalate was noted in the case of red clover and vetch whereas it actually stimulated the growth of California bur clover.

3. When 1 kgm. of pure Ottowa silica sand was used, which had been treated with concentrated hydrogen chloride and washed free of acid, the pH could be maintained constant provided about 200 cc. of Crone's solution was applied daily. The pH of the solutions used was altered with normal hydrogen chloride and sodium hydroxide.

4. The results of the comparative legumes tests on sand cultures show the following gradient of tolerance of low to high acidity, red clover, vetch, seredella, California bur clover, spotted bur clover, and velvet bean.

5. Results from the first series of the experiment tend to show that the vetch and bur clover plants can grow on a decidedly acid solution, provided nitrates are present. If, however, nitrates are not present but when the plants were inoculated such good growth in an acid range was not noted.

6. When the pH of soil was changed by the addition of an acid or base, the hydrogen ion was approximately maintained over a period of six and eight weeks provided the soil had first come to an equilibrium.

7. The best growth of most legumes was produced at a soil reaction of pH 6.0–6.8. When sodium hydroxide was used to change the reaction it destroyed the physical properties of the soil but some plants, particularly sweet clover, seemed to grow better when sodium hydroxide was used to change the reaction than when calcium hydroxide was used under similar hydrogen ion concentrations. This was particularly true when the soil was brought to a pH of 7.2 and 7.4.

8. The following range of acid tolerance was noted among legumes when grown on soil, ranging from high to low acidity: seredella, subterranean clover, vetch, bur clover, Austrian field pea, soybean, Canadian field pea, crimson clover, Hubam clover, and biennial white sweet clover.

REFERENCES

(1) Bauer, L. D. 1927 Factors affecting the hydrogen-ion concentration of soils. *Soil Sci.* 23: 399–414.

(2) Breaux, C., and Pren, J. 1927 The lime requirement of acid soils, and the slow reappearance of acidity after saturation with lime. *Compt. Rend. Acad. Sci.* 184: 1583–1585.

(3) Bryan, O. C. 1922 The effect of different reactions on the growth and nodule formation of soybeans. *Soil Sci.* 13: 271–302.

(4) Bryan, O. C. 1923 Effect of acid soils on nodule forming bacteria. *Soil Sci.* 15: 37.

(5) Bryan, O. C. 1923 Effect of reaction on growth, nodule formation, and calcium content of alfalfa, alsike clover, and red clover. *Soil Sci.* 15: 23.

(6) Coville, F. V. 1913 The agricultural utilization of acid lands by means of acid tolerant crops. U. S. Dept. Agr., Bur. Plant. Indus. Bul. 6.

(7) Dustiman, R. R. 1924 Effect of potassium acid pthalate on early growth of tomato. *Bot. Gaz.* 77: 419–431.

(8) Fred, E. B., and Loomis, N. E. 1917 Influence of hydrogen-ion concentration of medium on the reproduction of alfalfa bacteria. *Jour. Bact.* 2: 629–633.

(9) Fred, E. B., and Davenport, A. 1918 The influence of reaction on the nitrogen assimilating bacteria. *Jour. Agr. Res.* 14: 317–336.

(10) Hartwell, B. L., and Pember, F. R. 1918 The presence of aluminum as a reason for difference in the effect of so-called acid soil on barley and rye. *Soil Sci.* 6: 259–277.

(11) Hoagland, D. D. 1917 The effect of the hydrogen and hydroxyl-ion concentration on the growth of barley seedlings. *Soil Sci.* 3: 547–560.

(12) Joffe, J. S. 1920 Influence of soil reaction on the growth of alfalfa. *Soil Sci.* 10: 301–307.

(13) Loew, O. 1903 The physiological rôle of mineral nutrients in plants. U. S. Dept. Agr., Bur. Plant Indus. Bul. 45.

(14) Loew, O., and Aso, K. 1907 On physiologically balanced solutions. *Tokyo Imp. Univ. Col. Agr. Bul.* 7: 395–409.

(15) McCall, A. G., and Richards, P. E. 1918 Mineral food requirements of the wheat plant at different stages of its development. *Jour. Amer. Soc. Agron.* 10: 127.

(16) McIntire, W. H. 1921 Nature of soil acidity with regard to its quantitative determination. *Jour. Amer. Soc. Agron.* 13: 137–161.

(17) MIRASOL, J. J. 1920 Aluminum as a factor in soil acidity. *Soil Sci.* 10: 153–218.
(18) MIJOHE, K. 1916 The toxic action of the soluble aluminum salts upon the growth of the rice plant. *Jour. Biol. Chem.* 25: 23–28.
(19) PANTONELLI, E. 1915 Über Ionen Aufnohme. *Jahrb. Wiss. Bot. (Pringsheim)* 56: 689–733.
(20) PIERRE, W. H., AND PARKER, P. W. 1927 The use of collodian sacks in obtaining clear soil extracts for the determination of the water constituents. *Soil Sci.* 23: 13–32.
(21) RUNK, C. C. 1927 Annual report of director. Del. Agr. Exp. Sta. Bul. 152.
(22) RUNK, C. C. 1925 Hydrogen-ion concentration, buffer action, and soil type as a guide to the use of lime. *Jour. Amer. Soc. Agron.* 17: 345–353.
(23) TARR, L. W., AND NOBLE, S. C. 1922 The effect of hydrogen-ion concentration upon the growth of seedlings. Del. Agr. Exp. Sta. Bul. 131.
(24) THERON, J. J. 1924 Influence of reaction on interrelation of the plant and the culture medium. *Univ. Cal. Pub. Agr. Sci.* 4: 413–444.
(25) TRUOG, E. 1918 Soil acidity: I. Its relation to the growth of plants. *Soil Sci.* 5: 169–195.
(26) TRUOG, E. 1922 The feeding power of plants. *Science* 56: 1446; 294–298.

PLATE 1

VETCH PLANTS GROWN IN SAND CULTURE SOLUTIONS MAINTAINED AT DIFFERENT pH VALUES

FIG. 1. Inoculated, received a minus nitrogen culture solution. Solutions 25 = pH 4.5, 23 = pH 5.8, 27 = pH 7.0.

FIG. 2. Uninoculated, received a plus nitrogen culture solution. Solutions 22 = pH 4.5, 24 = pH 5.8, 28 = pH 7.0.

Fig. 1

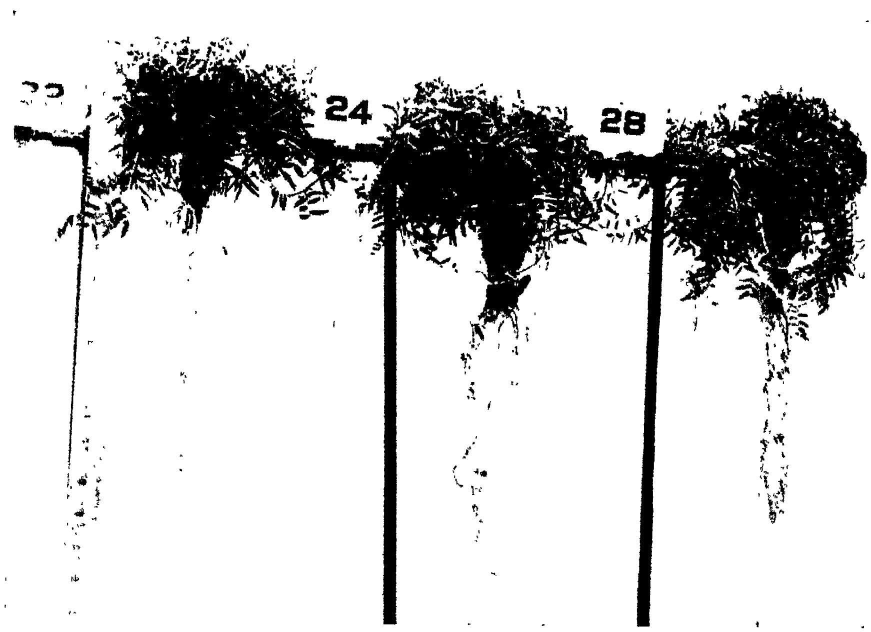


Fig. 2

PLATE 2

LEGUME PLANTS GROWN IN SAND CULTURES MAINTAINED AT THE pH INDICATED. INOCULATED PLANTS RECEIVED THE NITROGEN IN THE CULTURE SOLUTION

FIG. 1. Velvet bean.
FIG. 2. Seredella.


4.5
4.5
5.8
5.8
7.0
7.0
Inoc.
Uninoc
Inoc.
Uninoc.
Inoc.
Uninoc.

FIG. 1


4.5
Inoc.
4.5
Uninoc.
5.8
Inoc.
5.8
Uninoc.
7.0
7.0
Uninoc.

FIG. 2

PLATE 3

LEGUMES GROWN ON SOIL, THE H-ION CONCENTRATION OF WHICH WAS ADJUSTED AS INDICATED

FIG. 1. Canadian pea.
FIG. 2. Austrian pea.
FIG. 3. Biennial white sweet clover.
FIG. 4. Spotted bur clover. Note *L* indicates $Ca(OH)_2$ used to change soil reaction.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

AUTHOR INDEX

SUBJECT INDEX

ILLUSTRATIONS

PLATES

TEXT-FIGURES

A Study of the Nature of the Nitrogenous Compounds in Fungous Tissue and Their Decomposition in the Soil

The Influence of the Replaceable Bases on the Soil Solution Formation in Mineralized Soils

The Influence of Manganiferous Soils on the Accuracy of the Quinhydrone Electrode

The Measurement of "Suction Forces" in Soils

AN IMPROVED SOIL-SAMPLING TUBE

RELATION OF TEMPERATURE TO THE AMOUNT OF NITROGEN IN SOILS

A CRITICAL STUDY OF THE INFLUENCE OF SOIL TYPE ON THE CALCIUM AND MAGNESIUM CONTENT AND OTHER PHYSIOLOGICAL CHARACTERS OF THE ALFALFA PLANT

REPAIR OF SOIL FILTER TUBES

MICROBIOLOGICAL ACTIVITIES IN THE SOIL OF AN UPLAND BOG IN EASTERN NORTH CAROLINA

THE EFFECT OF MOISTURE CONTENT AND CROPPING ON EXCHANGEABLE CALCIUM AND MAGNESIUM, WITH PARTICULAR REFERENCE TO RICE SOIL

SOME INFLUENCE OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: I. HISTORICAL AND INTRODUCTORY